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FIG. 4.—*Splenic window.* Cat. In this photograph the outline of the spleen can be clearly seen. Omentum and coils of intestine are apparent to the right of the picture.

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AN INVESTIGATION OF THE IMMEDIATE EFFECTS OF X-RAYS ON LIVING ANIMAL TISSUES.

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Received for publication September 17th, 1925.

THE elucidation of the mechanism whereby X-rays influence living tissues may be greatly facilitated if an immediate indication of their operation can be obtained. The desirability of investigation of such changes is emphasized by the fact that the bulk of the previous work has concerned itself with delayed manifestations. Indeed, it may be said that almost all the biological changes produced by the X-rays are apparent only after the lapse of a latent period from the time of irradiation. Histological changes reveal themselves usually only after an interval measured in days or even weeks. Thus, although the diminished activity produced by X-rays on peroxidases appears immediately after an exposure of 1 hour, the disappearance of mitotic figures in tissue-cultures of chicken choroid reveals itself in 80 minutes after appropriate exposure, and the familiar cutaneous burn is delayed for some weeks.

Consider now the antithesis presented by ultra-violet light, which completely destroys the same enzyme, kills the same tissue-culture and inflames the skin in a period measurable in minutes.*

The significance of the above contrast is apparent when the possibility of this ultra-violet radiation arising in the tissues as a fluorescence excited by the X-rays is considered. The radiations which have access to the tissues may be not only the primary and secondary X-rays, but also fluorescent ultra-violet light lying within the long wave-length region above mentioned.

* For this discussion the wave-length of X-rays may be taken as between 1·0 and 0·15 Å.U., whilst the ultra-violet light may be arbitrarily considered as having a wave-length ranging from 3797 to 400 Å.U.

It has been shown (Perrine, 1923) that an ultra-violet fluorescence can be excited in crystals of NaCl, KCl, and several similar substances by means of X-rays. The possibility of bone acting in the same way has to be considered, although calcium oxide does not show the effect.*

These effects manifested by solid NaCl may, for excitation by X-rays, be similarly produced in solution, and in this way changes may be induced in the living cell. One would thus expect the almost immediate response characteristic of ultra-violet radiation.

A point of some interest in this connection is that crystals of NaCl, KCl, and in fact of the chlorides of all the alkali metals in a highly purified condition change colour when irradiated by X-rays for some 3 or 4 hours. The authority cited considers that it is "not unlikely that these colour changes are due to the reduction of a small fraction of the chloride into the metal concerned and free chlorine." The view taken is that there is a continuous decomposition of the salt with resulting fluorescence. This may, perhaps, afford an explanation of the efficacy of solutions of NaCl in the treatment of X-ray sickness. It is known, moreover, that the blood of patients suffering from this sickness contains less than the normal quantity of NaCl (Holzknecht).

The striking results obtained in cats and rabbits by Swann (1924) indicate the possibility of producing by X-rays immediate changes which are easily recognizable. This worker observed that "X-rays cause a gradual and regular fall of mean blood-pressure" accompanied by an initial stimulation of respiration followed by a depression, the final respiratory effect resembling that produced by morphine. It is suggested that this fall of blood-pressure, the onset of which occurred within a few minutes of the commencement of irradiation, was due to the production of toxic products. His opinion was that these products were not bodies of the nature of histamine.

Presuming, however, that the changes described are due to a chemical substance liberated into the circulation, the necessary photo-chemical relations governing its formation must assist in determining the mode by which the effects are produced. It was from this standpoint that the present series of experiments were performed, for X-rays, in the production of photochemical reactions, are distinguished by the absence of a threshold value below which no effect is produced (Glocker and Traub, 1921), whilst ultra-violet light, the possible production of which in the tissues has been considered, would display a threshold value. Thus if prolonged irradiation of an animal with X-rays of very feeble intensity could not produce a depressor substance yielded by the same quality of rays of a greater intensity, it would seem that its appearance cannot be ascribed to the photochemical action of X-rays. From these premises it should be possible to deduce the existence or otherwise of comparatively long wave-length fluorescence in the tissues.

EXPERIMENTAL.

In this connection it was at first obviously essential to determine if under any conditions of irradiation by X-rays a depressor substance appeared in the

* Chemically pure barium sulphate fluoresces in the ultra-violet when excited by X-rays—a point of some interest in view of the use of this salt in diagnostic radiography.

circulation of an experimental animal. For this purpose an intact unanæsthetized rabbit was subjected to irradiation for 7 hours, a gas tube being employed with a current of 2 milliampères and equivalent spark-gap of 12 cm., the exposure being interrupted for a few minutes at intervals of about a quarter of an hour. Target to skin distance was 13 cm.; no part of the animal was protected, a layer of cardboard 0·5 mm. in thickness (the bottom of the box containing it) alone intervening between it and the tube.

At the end of this irradiation blood was collected from the ear vein, heparin being used to prevent clotting. Blood from a normal animal was collected as a control. From a third animal a blood-pressure tracing was taken from the carotid. Five c.c. of blood from the normal rabbit were first injected into the ear vein, with no effect other than a momentary fall of blood-pressure of 5 mm., the pressure immediately returning to its former value. This small change is to be ascribed to the act of injection, as it occurred also upon the injection of the same quantity of normal saline. Five c.c. of blood from the irradiated animal were then injected. This also produced an identical effect. The conclusion was drawn that there was no depressor substance present. It was then thought that perhaps an anæsthetic such as urethane might be concerned in the production of such a substance. The great fall of pressure noted by Swann (1924) was obtained in animals so treated.

Accordingly an animal anæsthetized with urethane was irradiated for 3 hours with tube conditions the same as above, and a heparinized blood sample tested in the same way. Again no fall in pressure was noticed. The fact that animals after prolonged irradiation appeared to be in normal condition suggested that they had not suffered any considerable diminution in blood-pressure such as has been previously described. Moreover the occurrence of death in a rabbit after so short a time as 10 minutes, as described by Swann, indicated that some factor in addition to the X-rays was in operation. In this worker's experiments entirely unprotected animals were placed within one or two inches of the X-ray tube, and indeed in the case exhibiting the most marked changes of blood-pressure and respiration the skin of the animal was only half an inch away from the glass of the tube.

Under these conditions it is apparent that considerable electrical leakage through the animal from the X-ray tube and the high tension leads was inevitable, especially as the animal was laid on a copper operating table.

To obviate this possible source of error an electrical screen consisting of a metal box with a window of aluminium foil 0·005 mm. thick was arranged, the window being immediately above the animal. In this way it was hoped to place the tube in close proximity to the animal, and yet to avoid the possibility of electrical discharges occurring through it. Full intensity of X-radiation was thus nevertheless secured.

A urethanized rabbit was placed in this electrical screen, and a record of blood-pressure taken from its carotid artery. The rabbit was then subjected to continuous irradiation for half an hour, at the end of which time the blood-pressure, instead of showing the expected fall, had risen to 10 mm. (a continuous tracing was made of the blood-pressure). The electrical screen was then removed, and the animal subjected to a further irradiation of half an hour. During this period there was a slight continuous fall (Fig. 1).

Though this experiment has been repeated six times, in no case could the fall of blood-pressure described by Swann be obtained.

It was found impossible to cause any marked fall of blood-pressure even when the electrical screen was removed, though the tracing depicted in Fig. 1 demonstrates a slight fall in pressure dating from the removal of the screen.

From these experiments it is evident that no indication of a physiological action of X-rays can be obtained from observations on the blood-pressure. This is seen from Fig. 3 also.

FIG. 1.—*Blood-pressure tracing.* Rabbit. Urethanized. At *a* the irradiation was commenced with the aluminium box protection in position. This box was removed at *b*. Half an hour of continuous irradiation between *a* and *b*; half an hour between *b* and the end of the tracing. Equivalent spark-gap = 12 cm. Current = 1·5 milliampères. Distance of target to skin = 15 cm.

Respiratory Effects.

A very marked respiratory effect has also been described. Fig. 2 shows the result obtained from a rabbit on the respiratory depth and rate. The animal was anæsthetized with urethane and the tracing made from a tracheal canula. The animal was subjected to 1 hour of continuous irradiation; part of the time the electrical screen was interposed.

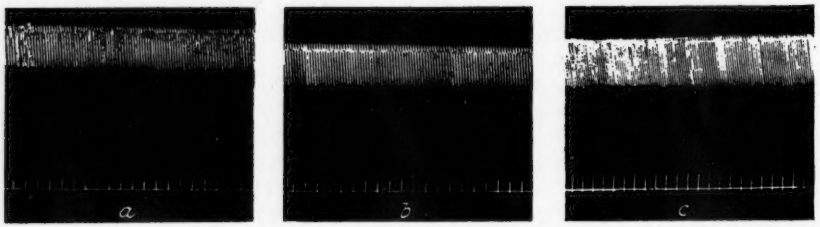

FIG. 2.—*Respiratory tracing.* Rabbit. Fig. *a*, before the irradiation. Fig. *b*, after irradiation for 1 hour. Fig. *c*, 2 hours after the cessation of the irradiation. Anæsthetic, urethane. Time-tracing in 6-sec. intervals. Equivalent spark-gap = 11 cm. Current = 1·5 milliampères. Distance of target to skin = 15 cm. (approx.).

From this tracing it is obvious that this amount of irradiation has no demonstrable effect on this function.

In this experiment the equivalent spark-gap was 11 cm.; current 1·5 milliampères; distance of target to skin 15 cm. (approx.).

A repetition of this experiment has also yielded no effects.

From the fact that these two easily determined physiological functions failed, under proper conditions, to yield any indication of an immediate effect

of X-rays, it was decided to investigate the size of the spleen. This organ is stated to show a diminution in size a few hours after a therapeutic exposure to X-rays. The very interesting work of Barcroft has shown that the spleen is an organ capable of considerable alterations of size under appropriate physiological conditions.

For the purpose of observing this organ abdominal windows were inserted so that a good view could be obtained of most of the spleen.

The following was the technique for the insertion of these windows.

The procedure is in the main that described by Katsch and Borchers (1913), modified in certain particulars.

The animals used were young cats. Under ether and chloroform anæsthesia a rectangular area of abdominal wall, previously thoroughly depilated, was removed. The cuts were so arranged that the interval between cut skin borders and the cut muscle borders was about 1 cm. after allowing for the

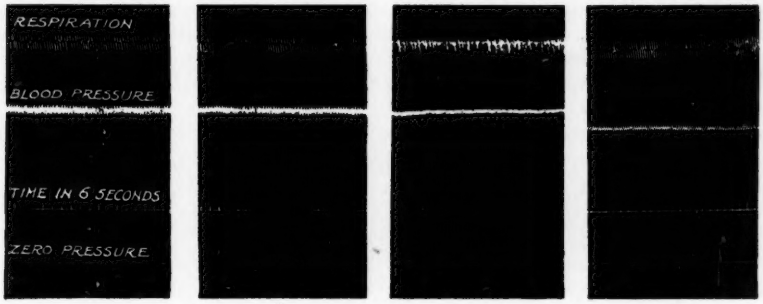

FIG. 3.—Cat. Urethane. Blood-pressure and respiration. Fig. *a*, before irradiation. Fig. *b*, 5 minutes after the beginning of irradiation. Fig. *c*, 15 minutes after the beginning of irradiation. Fig. *d*, 30 minutes after the beginning of irradiation. The cat had pronounced Cheyne-Stokes respiration before the beginning of the experiment and had just recovered when the irradiation was begun. The respiratory variations are thought to be due to the effects of the anæsthetic (compare Fig. 2). Equivalent spark-gap = 13 cm. Current = 1·5 milliampères. Distance of target to skin = 15 cm.

elastic retraction of these structures. A celluloid plate (cut photographic film freed from gelatine and sterilized in mercuric chloride solution) was then sutured to the muscle by a series of close interrupted silk sutures. Complete hæmostasis was essential. The celluloid overlapped the muscle by at least 1 cm. After suturing the plate to the muscle the cut edge of the skin was sutured to the edge of the celluloid plate. Thus there is no " dead " space left in which sepsis could most easily occur. By this procedure the skin does not overlap the celluloid plate. The inadvisability of allowing the skin to overlap the plate and of the use of celloidin as an agent for sealing the skin and plate together has been previously described (Florey and Carleton). A good way of sealing the junction of skin and plate has been found to be the application of small pieces of cotton-wool soaked in tinct. benzoini co. B.P., and after this has dried to coat the joint with collodion.

At the conclusion of the operation the cat was covered in a thick layer of cotton-wool and then immobilized by wrapping in plaster-of-Paris bandages, the head alone being left free. By this means the violent movements associated with the recovery from the anæsthetic were controlled. After the lapse of about 10 hours the plaster was removed.

In this way animals were prepared in which the spleen and intestines could be watched under approximately normal conditions (Fig. 4).

The irradiations were usually done on the third day after the operation, by which time the animal was so far recovered that it was able to walk around the room, to jump up on one's shoulder, and indeed it presented no marked change attributable to the operation.

A window over the heart was also inserted. This was done in a decerebrate cat. The animal was kept alive by means of artificial respiration during the insertion of the celluloid. Portions of the lung overlapping the heart were ligated and removed. As soon as the window was made airtight and most of the air removed by suction through a tube, the animal was allowed to resume its normal breathing. Under these conditions the animal lived $4\frac{1}{2}$ hours with a very active condition of the heart.

For purposes of comparison the outline of the spleen, before irradiation, was marked on the celluloid window. The animal was then subjected to 4 hours' continuous irradiation with tube conditions as follows: Equivalent spark-gap, 11 cm.; current, 1·5 milliampères; distance of target to skin, 15 cm.

At the end of the irradiation the animal was examined, but no detectable difference in splenic volume was present. Moreover, the animal appeared to be perfectly well after it was put back in its cage.

That the spleen of this animal was capable of contraction was shown when the cat was subsequently killed by chloroform. The spleen under these conditions contracted to about two-thirds of its former volume.

The decerebrate animal prepared with a transparent window above the heart was subjected to $1\frac{3}{4}$ hours' continuous irradiation, but during that whole period no difference could be detected in its action. Certainly no fibrillation occurred, as was found by the worker previously mentioned.

DISCUSSION.

The foregoing experiments fail to demonstrate any immediate effects from X-radiation which might afford an insight into the mode of action of X-rays on living tissue.

It is obvious that X-rays produce photochemical changes in the body, either directly or indirectly, and any index of such a change should assist in solving the problem in question.

It must be concluded, however, that a study of blood-pressure, respiration or splenic volume does not yield the desired indication.

It would appear that the positive results obtained by Swann are to be explained as being due to high-tension electrical discharges. All his effects on blood-pressure and respiration are similar to those obtained by placing a metal electrode over the thorax of an animal and then passing a high-tension discharge through it. In such conditions there is a fall of blood-pressure, a

cessation of respiration, and a terminal fibrillation of the heart. The commonest cause of death from electrocution is from ventricular fibrillation.

The explanation offered for the lack of fall of blood-pressure in our animals, even when not electrically screened, was that in all cases the tube has not been placed so near the animal, and it was impossible to reproduce the precise conditions with regard to electrical leakage occurring in Swann's experiments.

SUMMARY.

(1) The possibility of ultra-violet fluorescence excited within the tissues by X-rays is considered as a possible mechanism operating in the production of effects by X-rays.

(2) Contrary to the results obtained by previous workers, no immediate fall of blood-pressure or alteration in respiratory rate occurs during prolonged irradiation.

(3) It is suggested that previous results are explicable as due to electrical leakage, and not to the action of X-rays.

(4) A method for the insertion of abdominal windows is described.

(5) By means of these windows no changes could be observed in the spleen or other abdominal viscera.

We are indebted to Prof. J. N. Langley for kindly placing his X-ray apparatus at our disposal, and to Dr. T. S. P. Strangeways for lending us an X-ray tube. To Dr. M. B. R. Swann we are indebted for information concerning his experimental conditions.

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AMMONIA IN THE BLOOD OF EPILEPTICS.

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BISGAARD and Norvig (1920) and Norvig (1924) have found a great increase of ammonia in the blood of epileptics before the onset of fits. They employed, however, the method of ammonia estimation of Henriques and Christiansen (1916). This method gives much higher values than are given by the methods used by Nash and Benedict (1921) and by Parnas and Heller (1924), in which greater precautions are taken to avoid the breakdown of labile ammonia-forming substances in the blood. We have therefore estimated the ammonia in the blood of epileptics by a method devised by Luck and Seth (1925), which gives values in agreement with these latter methods. The values found are given in milligrammes of ammonia per 100 c.c. The normals and most of the other controls gave values below 0·10; we can therefore consider values above 0·10 abnormally high, and those above 0·20 much raised.

In spite of repeated attempts we succeeded in bleeding patients within six hours before a fit on only seven occasions. However, the interest of this aspect of the question was overshadowed by that of the effect of starvation and ketosis. In a control subject, a fat young woman in good health except for moderate sciatica, and in a patient with diabetic ketosis, the blood ammonias were very high; in another patient with diabetic ketosis the ammonia was high, but estimation was impossible owing to the formation of a precipitate, due to the large amount of acetone present. A similar increase occurred in the blood-ammonia of the epileptics who were starved (Nos. 18, 21, 22, 23, 24).

In four estimations made just after fits (Nos. 18, 19, 21, 24) the blood-ammonia was raised in three; no such increase was found in the normals after exercise, but it cannot be inferred from this that the increase of blood-ammonia is not due to the muscular exertion and insufficient aëration alone, for although the normal subjects probably did far more muscular work than a patient in a fit, they could not reproduce the cessation of and interference with respiration. Of the seven estimations before fits No. 24 and one of No. 23 were during starvation; the blood-ammonia was not higher than in other patients during starvation; No. 24 was high—this was immediately after a fit; No. 23 was slightly above normal on three occasions—two of these were shortly after fits; No. 21 was normal; No. 17 alone was raised without any cause. It appears from consideration of the whole table that high blood-ammonia may occur at times in epileptic patients, but not necessarily before fits; from the fact that high blood-ammonia occurs regularly in starvation, although fits are often much less frequent, it is impossible to consider high blood-ammonia as the cause of the fits.

TABLE I.—*Blood-Ammonia in Non-Epileptics.*

Case.	Date.	Blood-ammonia mgm. NH_3 per 100 c.c.	Remarks. (Plasma bicarbonate in c.c. CO_2 per cent. plasma.)
1. Normal	17.1.25	0·08	—
2. Normal	9.1.25	0·02	—
	14.1.25	0·07	After exercise (stair-running).
3. Normal	31.12.24	0·08	—
	15.9.25	0·04	After exercise ("knees-up" 4 minutes). Plasma bicarbonate 44.
4. Normal	16.2.25	0·08	—
5. Sciatica	7.1.25	0·08	—
	13.1.25	0·08	—
	27.1.25	0·22	Starved 11 days. Plasma bicarbonate 45. Blood-acetone bodies 0·11 per cent.
6. Thrombo-angeitis obliterans	1.1.25	0·04	—
7. Heart failure	31.12.24	0·09	Liver enlarged and painful.
8. Partial intestinal obstruction	5.1.25	0·05	—
9. Nephritis	2.1.25	0·12	Blood-urea 0·087 per cent.
10. Nephritis	2.1.25	0·03	„ 0·167 „
11. Nephritis	2.1.25	0·13	„ 0·228 „
12. Nephritis	5.1.25	0·06	„ 0·045 „
13. Nephritis	6.1.25	0·06	„ 0·54 „
14. Nephritis	17.1.25	0·09	„ 0·34 „
15. Diabetic coma	16.1.25	0·37	Plasma bicarbonate 14.

TABLE II.—*Blood-Ammonia in Epileptics. "No fits" implies none within 24 hours.*

Case.	Date and time.	Blood-ammonia mgm. NH_3 per 100 c.c.	Relation to fits.	Remarks. (Plasma bicarbonate in c.c. CO_2 per cent. plasma.)
16. Petit mal	31.12.25 —	0·10	No fits	—
	1.1.25 —	0·09	„	—
17. Grand mal	2.1.25 10.30 a.m.	0·41	Fit 2 hrs. later	Fits of moderate severity.
18. Grand mal	3.1.25 1.10 p.m.	0·06	Fit 1 a.m. 4.1.25	„ „
	5.1.25 10.55 a.m.	0·06	Fit early morning 6.1.25	—
	7.1.25 10.45 a.m.	0·09	Fits early morning 7.1.25, 8.1.25	—
	9.1.25 10.40 a.m.	0·06	No fits	—
	10.1.25 10.50 a.m.	0·14	„	—
	12.1.25 11.35 a.m.	0·10	Fit early morning, 12.1.25	—
	13.1.25 3.30 p.m.	0·25	In coma 15 mins. after onset of fit	—
	16.1.25 10.30 a.m.	0·06	No fits	—
	29.1.25 —	0·47	„	Starving since 20.1.25. Plasma bicarbonate 51. Acetone bodies 0·08 per cent.
	2.2.25 11.45 a.m.	0·44	3.35 a.m., 2.2.25	Supposed to be starving, but had probably broken fast. Plasma bicarbonate 67. Trace of acetone in urine.
	6.2.25 2.15 p.m.	0·16	Fits 1.30 and 2.30 a.m., 6.2.25	Normal diet from 3.2.25.

TABLE II.—*cont.*

Blood-Ammonia in Epileptics. "No fits" implies none within 24 hours.

Case.	Date and time.		Blood-ammonia mgm. NH_3 per 100 c.c.	Relation to fits.	Remarks. (Plasma bicarbonate in c.c. CO_2 per cent. plasma.)
19. Grand mal	13.1.25	10.45 a.m.	0·09	No fits	Fits of moderate severity.
	15.1.25	10.55 a.m.	0·06	,,	—
	28.1.25	11.50 a.m.	0·10	15 minutes after fit.	—
	4.2.25	11.50 a.m.	0·24	No fits before; left hospital same afternoon	—
20. Grand mal	14.1.25	10.45 a.m.	0·09	No fits	—
	15.1.25	10.55 a.m.	0·06	,,	—
21. Grand mal	16.1.25	3.30 p.m.	0·06	Fit at 8 p.m.	Fits of moderate severity.
	2.2.25	3.10 p.m.	0·055	None	—
	10.2.25	1.30 p.m.	0·188	In coma about 15 minutes after onset	—
	17.2.25	—	0·100	No fits	—
	23.2.25	—	0·065	,,	—
	2.3.25	—	0·085	,,	—
	10.3.25	—	0·326	,,	Starved from 2.3.25. Plasma bicarbonate 34. Blood-acetone bodies 0·145 per cent.
22. Petit mal	27.3.25	11 a.m.	0·064	No fits	—
	7.4.25	—	0·088	,,	—
	15.4.25	—	0·063	,,	—
	18.4.25	—	0·17	,,	Starved from 15.4.25 to 22.4.25.
	20.4.25	—	0·276	,,	Plasma bicarbonate 38. Blood-acetone bodies 0·155 per cent.
23. Grand mal	1.5.25	2.0 p.m.	0·11	Fits at 12.40 p.m. and 6.30 p.m.	Fits not severe.
	7.5.25	2.0 p.m.	0·095	Fits at 9.45 a.m. 5.50 and 7 p.m.	—
	16.5.25	11.50 a.m.	0·285	Fits at 8.30 a.m., 6.30 p.m.	Starved from 13.5.25 to 21.5.25. Plasma bicarbonate 61.
	21.5.25	11.40 a.m.	0·355	Fits at 9.15 a.m., 5.30 and 6 p.m.	Plasma bicarbonate 46. Blood-acetone bodies 0·07 per cent.
	30.7.25	2.0 p.m.	0·11	Fit at 2.16 p.m., and 10 more before night	—
24. Grand mal	11.6.25	11.30 a.m.	0·246	No fits	Severe fits.
	13.6.25	11.15 a.m.	0·16	Immediately after clonic movements ceased. Another fit 2.10 p.m.	—
	19.6.25	1.45 p.m.	0·415	Fit at 6.50 a.m.	Starved 13.6.25 to 23.6.25. Plasma bicarbonate 34. Blood-acetone bodies 0·175 per cent.
25. Grand mal	18.4.25	11 a.m.	0·17	Comatose after status epilepticus	—
	20.4.25	11 a.m.	0·154	Much better. No fits since 18.4.25	—

CONCLUSIONS.

An increase of blood-ammonia is found in ketosis, whether occurring in starvation or in diabetes mellitus.

High blood-ammonia does not occur invariably before epileptic fits, and is not the cause of epileptic fits.

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THE RELATION BETWEEN THE EXTENT OF MITOSIS SEEN IN CHICK FIBROBLASTS AND THE CONCENTRATION OF EMBRYONIC TISSUE EXTRACTS PRESENT IN THE TISSUE CULTURE MEDIUM.

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In 1921 Ebeling (1921) introduced a method for the quantitative measurement of growth in tissue cultures which has been applied with much success. It suffers from the drawback, however, that a strain of fibroblasts has to be maintained in the laboratory—a procedure involving considerable additions to the labour of the investigation. A second method based on the extent of mitosis as the criterion of growth has been suggested by Lambert (1914), Price-Jones and Mottram (1914), and again by Mottram (1925), all of whom emphasize the possibility of confusion between true growth and mere emigration.

The great importance of embryonic extracts for the growth of tissue fragments has been stressed by Carrel (1924), who states that "fibroblasts

and epithelial cells may be considered as in a true state of cultivation only when embryonic tissue juices are present in the medium." Not only does he find it essential for growth, but the extent to which this took place was in the lower grades of dilution of the extract closely related to the concentration in which it was present.

In consequence the following investigation was made to find out whether the frequency of mitosis, and so inferentially the growth in cultures prepared as described below, had any similar relation to the concentration of embryonic extracts in the nutrient medium. Should such a relation hold good it should correlate the method of Mottram with that of Ebeling, and so provide a simple means for the estimation in various nutrient media of those substances which activate the growth of tissues.

METHOD.

The technique employed was the ordinary hanging-drop method of cultivation. The cells used in the experiments were fibroblasts derived from the hearts of embryo chicks of between 9 and 12 days' incubation. Ebeling has found that these cells are second only to those of a permanently cultivated strain of fibroblasts in the uniformity of the rate of multiplication. They also possess the great advantage of easy cultivation *in vitro*.

The medium employed was obtained by suspending the finely-minced fragments of an entire chick embryo of 9 to 12 days' incubation in about 10 c.c. of the saline solution devised by Pannett and Compton (1924). This suspension was allowed to stand for some half hour with occasional shaking, was then centrifuged, and the slightly opalescent supernatant fluid drawn off with a pipette. The medium was then ready to be used.

For each series of experiments some 40 to 50 cultures of heart-muscle fragments were set up in this medium and were cultivated in it for about 48 hours. At the end of this period they were examined under the microscope, and those showing good halos of cells surrounding the original implanted fragment were selected and set aside while the remainder were rejected. Every effort was made to subject these slides to a uniformity of conditions both inside and outside the incubator.

In this way a number of cultures were obtained having roughly the same numbers of cells which had emigrated and were capable of subsequent microscopical examination. These selected slides were then re-cultured after preliminary washing in the various media whose activity it was desired to examine. At the end of some 15 to 20 hours of this second cultivation they were removed from the incubator and rapidly fixed in Bouin's medium and stained with iron alum. This process requires to be carried out quickly, since the total duration of mitosis in this variety of cell lasts only 30 to 40 minutes. After staining and mounting, the preparations were examined under an oil-immersion lens and the number of mitoses in each culture was counted.

RESULTS.

The effects of three grades of dilution of the embryonic extract medium were studied. The most concentrated grade was composed of the medium as it was initially prepared; the other two by dilution of this with Pannett and

Compton's saline in the proportions of 2 parts extract to 1 part of saline and 1 part extract to 2 of saline. In this way three media were made up containing the active principles in the proportion of 3, 2 and 1. All three media appear to be comparable with the more dilute grades used by Carrel and Ebeling (1921).

The following tables represent the frequency of mitosis in cultures under these conditions. For series A the number contained in each culture is given, for the others merely the averages deduced from the entire series.

Series A: Culture number.	Number of mitoses in cultures.		
	Original extract.	2 extract, 1 saline.	1 extract, 2 saline.
1	43	28	16
2	20	28	14
3	48	24	5
4	35	28	21
5	22	49	8
6	47	39	22
7	27	11	5
8	51	25	23
Average	37	29	14

Further series were set up under similar conditions of dilution:

Series.	Number of mitoses in cultures (averages).		
	Original extract.	2 extract, 1 saline.	1 extract, 2 saline.
B	55	43	Infected
C	48	37	29
D	113	70	41

These results indicate that mitosis is more active in the more concentrated media. The relationship between activity and concentration cannot be represented strictly by a linear function, though in certain of the series it approximates to it. In general, the same results have been observed as those found by Carrel, namely that in the more dilute grades the active principles are more than proportionately potent, and that the activity falls away somewhat in the highest grade. Of the theoretical aspects of this relationship it is as yet too early to speak.

Whether these substances present are used as sources of nutrition or can be more properly described as stimulants for the cell is unknown. It would seem, however, that these cells require a period of time between successive mitoses which additional concentrations of these nutrients or stimulants are increasingly unable to cut down until an ultimate limit is reached beyond which the intermitotic period cannot be shortened.

Finally, it would seem that within the crude limitation necessarily

associated with investigations in a field as yet so little developed, this method may be used in the calibration of nutrient media, and that so long as dilute extracts are used the relation between concentration and mitosis may without gross error be regarded as rectilinear.

In conclusion, brief mention may be made of some preliminary experiments in which this method of estimating growth has been employed. Embryonic tissue extracts have been dialysed against the saline of Pannett and Compton to see whether any mitosis-exciting substances were capable of diffusing through. The membranes were made of collodion, with special attention to the points emphasized by Gates (1921). Further, the substances which had passed through into the outer fluid were subsequently heated to over 100° C. in the autoclave for 10 minutes. In no case did the outer solution give a biuret reaction.

The following table summarizes the results:

Series.	Average number of mitoses in cultures.			
	Inner solution.	Outer solution.	Saline only.	Heated outer solution.
1 . .	22 .	10 .	— .	—
2 . .	25 .	6 .	2 .	7
3 . .	7 .	— .	1 .	7

It would appear from these results that mitosis-activating substances are capable of passing through a dialysing membrane, but in general they are not present in as great concentration outside as inside. Whether the cells could continue to survive on such a protein-free medium could only be determined by prolonged experiments. It would further appear that once separated from the proteins present in embryonic extract the mitosis-activating substances exhibit a greatly increased resistance to destruction by heat.

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PLASMA-LIPOIDS IN RENAL DISEASE.

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As far back as the time of Bright it was known that, in certain cases of nephritis, the blood-serum presented a milky appearance—an appearance which was rightly attributed to an increase of fatty substances in the blood. Bloor (1917) gave an excellent abstract of the literature on the subject to that date. In many cases of chronic parenchymatous nephritis, particularly that group which he characterizes as nephroses, Epstein (1922) has shown that there is a significant increase in the plasma-lipoids, and has laid special emphasis on the cholesterol increase. He examined the blood-lipoids in 100 cases of renal disease and allied conditions, and found that in those of chronic parenchymatous nephritis—chiefly in the œdematous stage—there was an increase in the blood-lipoids, whereas in the uræmic cases with nitrogen retention the lipoids were diminished. On the other hand, Denis (1917), in an investigation of forty-five similar cases, found a noteworthy increase in the cholesterol content of the blood in only one.

In view of this divergence of opinion and the fact that the changes in the cholesterol content have been emphasized seemingly to the exclusion of the other lipoids, Prof. Arthur Ellis suggested that this present work be undertaken. It was hoped that it might also be possible to determine whether a relationship exists between the lipoid content of the kidneys post-mortem and the presence of hyperlipæmia during life. If such a relationship could be established, it would suggest that the kidney lipoid is not, as is generally supposed, a fatty degeneration, but merely a lipoid infiltration resulting from the high fat content of the blood. As will be seen, our results support the contention of Epstein that in chronic parenchymatous nephritis the blood-lipoids are increased. No example of chronic interstitial nephritis occurred in the series.

Methods Employed.

No attempt was made to standardize the diet of the patients examined. They were all on the usual ward nephritic diet, which was low in salt and proteins. Oxalated blood was used. The blood sample was removed from 3 to 4 hours after breakfast from an ante-cubital vein, stasis being avoided as far as possible. All determinations were made on blood-plasma. The cholesterol and fatty acid content was determined by the method described by Bloor, Pelkan and Allen (1922). The lecithin was determined as lipoid phosphorus. The methods described by Baumann (1924) and by Briggs (1924) were combined. The reagents used were those recommended by Briggs, and his method of colour development was followed. The digestion

TABLE I.—*The Blood Lipoids and Plasma Protein in 18 Cases of Renal Disease and 5 Normal Controls.*

Name.	Date.	Diagnosis.	Character of serum.	Cholesterin, mgm. per 100 c.c.	Fatty acids, mgm. per 100 c.c.	Lecithin, mgm. per 100 c.c.	Fibrin, gm. %.	Albumen, gm. %.	Globulin, gm. %.	. .	Blood-urea, gm. %.	Phenol-sulphone-phthalein, 2 hrs. %.	Urea concentration.
1	1.10.24.	Normal	Clear	216	338	166	0·27	5·12	2·07	2·4	—	—	—
2	6.10.24.	„	„	143	400	175	0·29	4·72	2·05	2·5	—	—	—
3	6.10.24.	„	„	161	442	179	0·28	5·17	2·40	2·1	—	—	—
4	1.10.24.	„	„	231	400	167	—	—	—	—	—	—	—
5	1.10.24.	„	„	189	556	202	0·37	4·95	2·62	1·8	—	—	—
6	16. 7.24.	Chronic parenchymatous nephritis	„	304	678	185	0·33	1·53	1·74	0·9	0·037	41	243 c.c., 0·48 % 175 c.c., 0·54 %
	29. 8.24.	—	„	475	593	190	0·57	3·22	3·3	0·98	0·037	26	81 c.c., 0·84 % 37 c.c., 0·86 %
7	15. 9.24.	Chronic parenchymatous nephritis	Slightly buffy	239	490	155	0·62	2·65	2·45	1·08	0·085	16	150 c.c., 0·84 % 120 c.c., 0·96 %
8	24. 9.24.	Ditto	Clear	239	480	156	0·50	2·53	2·57	0·98	0·021	72	100 c.c., 1·80 % 73 c.c., 2·46 %
9	31. 7.24.	„	Creamy	276	923	—	0·67	1·12	1·27	0·8	0·093	15	2 hrs., 2·34 %
	19. 8.24.	—	„	422	952	314	0·80	1·60	2·00	6·8	0·114	—	46 c.c., 1·32 % 52 c.c., 1·56 %
	10. 9.24.	—	„	667	1176	278	0·41	1·63	2·04	0·8	0·093	—	—
10	28. 7.24.	Chronic parenchymatous nephritis (After decapsulation)	„	624	1053	380	0·50	1·81	2·34	0·8	0·063	25	48 c.c., 1·44 % 34 c.c., 1·56 %
	6. 8.24.	—	Clear	395	857	318	0·66	1·90	1·61	1·1	0·157	—	42 c.c., 1·02 % 32 c.c., 1·20 %
	13. 8.24.	—	„	253	513	249	0·64	2·04	1·28	1·6	0·171	—	—

11	22. 7.24.	Chronic parenchymatous nephritis	Clear	289	1136	350	1·04	1·36	2·77	0·5	0·024	37	92 c.c., 2·46 % 69 c.c., 2·82 %
12	8. 8.24.	Ditto	„	376	561	278	1·10	1·18	2·32	0·5	0·063	36	108 c.c, 1·44 % 85 c.c., 1·62 %
	17. 9.24.	—	„	303	500	156	—	—	—	—	0·051	—	—
13	17. 9.24.	Acute nephritis	„	118	369	109	—	—	—	—	0·165	20	165 c.c., 1·32 % 130 c.c., 1·50 %
14	26. 8.24.	Ditto	„	131	381	159	0·37	3·55	4·16	0·8	0·033	—	—
15	1. 9.24.	Toxæmia of pregnancy	„	286	492	200	—	—	—	—	0·025	70	43 c.c., 3·24 % 83 c.c., 3·18 %
16	25. 7.24.	Toxæmia of pregnancy; pyelitis	„	108	524	143	0·68	2·37	2·90	0·8	0·399	5·4	23 c.c., 1·02 % 57 c.c., 1·26 %
	13. 8.24.	—	„	—	—	—	0·68	6·1	—	—	0·180	—	—
	8. 6.25.	—	—	—	—	—	—	—	—	—	0·137	—	—
17	6.10.24.	Congenital cystic kidney	„	146	429	109	0·53	3·60	4·20	0·86	0·375	—	—
18	10.10.24.	Pyelo-nephritis; uræmia	„	163	533	113	0·49	4·70	3·20	1·47	0·240	—	—
19	9. 9.24.	Ditto	„	129	616	188	—	—	—	—	0·199	—	—
20	25. 8.24.	Urethral stricture; pyelitis	„	263	375	139	0·42	4·27	2·62	1·6	0·036	74	121 c.c., 1·32 % 92 c.c., 1·44 %
21	23. 9.24.	Arterial hypertension; heart failure	Clear; slightly jaundiced	159	411	114	0·40	5·06	2·77	1·8	0·045	49	75 c.c., 2·22 % 45 c.c., 2·82 %
22	12. 9.24.	Arterial hypertension	Clear	136	388	109	0·49	4·4	3·0	1·46	0·042	49	20 c.c., 1·98 % 16 c.c., 1·86 %
	7.10.24.	(Cardio-renal disease)	„	106	455	130	0·80	2·82	2·90	0·97	0·082	8	—
23	2. 7.24.	Pulmonary and renal tuberculosis	„	180	715	148	1·16	1·76	3·57	0·5	0·044	16	47 c.c., 1·50 % 94 c.c., 1·74 %

was accomplished by the use of seven drops of pure concentrated sulphuric acid and 0·2 c.c. of 30 per cent. hydrogen peroxide, more peroxide being added drop by drop when charring occurred. It was found that the colour development was better if, after digestion over the micro-burner, the contents of the pyrex tube were transferred to a glass evaporating dish and heated over a water-bath until only a few drops remained. The digested clear fluid was then returned quantitatively to the original pyrex tube for colour development.

The plasma proteins were determined by the method described by Wu (1922). The determinations of the plasma proteins were made by Dr. John R. Marrack, who has been good enough to allow me to incorporate them in this paper.

The phenolsulphonephthalein excretion was determined by the method of Rowntree and Geraghty (1910). The injection of the dye was subcutaneous and the appearance time was not noted. The blood-urea was determined by the method described by Myers (1914). The urea concentration test was done according to the method of MacLean and de Wesselow (1920).

Results.

The results of the investigation are given in Table I. For convenience of comparison the values obtained by several investigators of the lipoids and proteins of blood-plasma in normal persons are shown in Tables II and III.

TABLE II.—*Plasma-Lipoids.*

	Cholesterin, mgm. per 100 c.c.	Fatty acids, mgm. per 100 c.c.	Lecithin, mgm. per 100 c.c.
Average of five normal persons examined (Daniels)	208	427	178
Average values according to Bloor (1917)	230	390	200
Average values calculated from normals done by Hiller, Linder, Lundsgaard and Van Slyke (1924)	182	359	220

TABLE III.—*Plasma Proteins.*

	Total, protein, gm. %	Albumen, gm. %	Globulin, gm. %	A/G, ratio.
Normal values according to Epstein (1917)	7·4	4·662	2·738	1·702
Normal values according to Linder, Lundsgaard, Van Slyke and Stillman (1924)	6·73	4·11	2·61	1·6
Average of four normal persons examined by Dr. Marrack	7·577	4·99	2·285	2·18

In Table IV the maximum, minimum and average values of plasma-lipoids obtained in normal individuals, in patients with chronic parenchy-

matous nephritis and in patients with other renal disorders are extracted from Table I.

Examination of the tables reveals a definite increase in the plasma-lipoids of five out of the seven patients with chronic parenchymatous nephritis examined. In two instances no increase was found. The increase is most noticeable in the case of cholesterol but apparently occurs also with the other lipoids. Averaging the results it will be found that the average value for choleresterol in the patients with chronic parenchymatous nephritis was 80 per cent. above the average of normal individuals examined and 130 per cent. above the average found in patients with other kidney disturbances. The average value found for fatty acids was 81 per cent. above the average in normal individuals, and 65 per cent. above the average in other renal cases.

TABLE IV.—*Résumé of Results.*

	Cholesterin, mgm. per 100 c.c.	Fatty acids, mgm. per 100 c.c.	Lecithin, mgm. per 100 c.c.
Normals:			
Maximum	231	556	202
Minimum	143	338	166
Average	208	427	178
Chronic parenchymatous nephritis:			
Maximum	667	1176	380
Minimum	239	480	155
Average	374	770	251
All other cases:			
Maximum	286	715	200
Minimum	106	369	109
Average	163	467	137

The average value of lecithin was 41 per cent. above the average in normal individuals, and 83 per cent. above the average in the other kidney cases. These increases appear too great to be merely accidental. They seem to indicate a definite increase in the plasma-lipoids in chronic parenchymatous nephritis.

In the patients with renal conditions other than chronic parenchymatous nephritis, the normal average value for cholesterol was exceeded twice—once in a pregnant woman with albuminuria, Case 15, and again in a urinary tract infection, Case 20. Neither of these patients showed a really high level of cholesterol. The lecithin average normal level was also exceeded twice (Cases 15 and 19), but in both these instances the values obtained were within the average normal figure given by Bloor, and did not exceed the maximal value found by us in normal individuals. The average value for both cholesterol and lecithin was less in the cases of renal disease excluding chronic parenchymatous nephritis than in the normal individuals. The fatty acids, on the other hand, were greater in these cases than in normal individuals, in two instances the figures being definitely higher than the maximum finding in the

normals (Cases 19 and 23). One of these was a cachectic phthisical man with renal disease probably tuberculous in character, the other a fatal case of pyonephrosis.

In Case 10 it is interesting to note the fall in the level of the lipoids as the blood-urea rises. This phenomenon has been noted by Henes (1920) and others. Decapsulation was performed on this patient and also on Case 6. A small section of kidney-tissue was removed at each of these operations and microscopic sections made. The sections were so similar that it was practically impossible to distinguish one from the other. Both showed a moderate degree of diffuse fibrosis and abundant lipoid about the tubules.

Through the kindness of Dr. John R. Marrack, I have been able to add to this paper determinations of the plasma proteins of most of the cases examined. These determinations were made on the same blood samples as the lipoid determinations. In the cases of chronic parenchymatous nephritis studied the total protein values are invariably low and the albumen to globulin ratios are also low. The inversion of the albumen to globulin ratio is, however, not confined to the cases of parenchymatous nephritis. Though the cases of this type of kidney disease seem to have high lipoids and low plasma proteins, no definite relationship can be said to exist between these two blood constituents.

PROTOCOLS OF CASES.

Cases 1 *to* 5.—Normal individuals in good health.

Case 6.—Female. Diagnosis: Chronic parenchymatous nephritis. Admitted with ascites and much œdema. Urine: Sp. gr. 1020; albumen $\frac{1}{5}$ of volume; granular casts. Decapsulation was performed and followed by very marked improvement and eventually disappearance of symptoms. The first examination of the blood for lipoids was done two months after the decapsulation. Discharged. The patient still has albuminuria but has remained symptom-free for two months after operation. Section of kidney showed abundant lipoid about the tubules and moderate diffuse fibrosis.

Case 7.—Middle-aged female. Diagnosis: Chronic parenchymatous nephritis. Seven years' history of mild recurrent œdema. Blood-pressure remained pretty steadily 145, systolic, until a year ago; now 200, systolic. Generally worse. Palpable radial arteries and some cardiac enlargement. Urine: Low sp. gr.; albumen $\frac{1}{6}$ to $\frac{1}{3}$ of volume; granular casts, red and white blood-corpuscles. Died in uræmia. Post-mortem: Chronic parenchymatous nephritis.

Case 8.—Male, aged 27. Diagnosis: Chronic parenchymatous nephritis. Three years' history of headache, backache and blurred vision. Giddiness for twenty months. Two weeks' œdema and "asthma." On examination there was moderate generalized œdema, albuminuric retinitis. Blood-pressure, 130 systolic, 85 diastolic. No cardio-vascular change. Urine: Alkaline; sp. gr. 1010 to 1020; albumen $\frac{1}{4}$ to $\frac{1}{3}$ of volume; blood and granular casts.

Case 9.—Male, aged 24. Diagnosis: Chronic parenchymatous nephritis. The patient was admitted to the hospital with a history of œdema and vomiting of six weeks' duration. Physical examination revealed massive œdema and ascites. Blood-pressure, 142 systolic, 105 diastolic. The œdema did not decrease after admission. The urine examination: Sp. gr. 1022 to 1040, acid; albumen, $\frac{2}{3}$ to $\frac{9}{10}$ of volume; many granular casts. Paracentesis was performed and fluid removed from the abdomen. Southey tubes inserted in lower extremities resulting in copious drainage. The œdema fluid showed only traces of lipoids. After two months the œdema had greatly decreased and the patient was much improved. He, however, subsequently relapsed and again became grossly œdematous.

Case 10.—Female. Diagnosis: Chronic parenchymatous nephritis. Admitted to the hospital with weakness and moderate œdema. Systolic blood-pressure, 150. Urine: Albumen $\frac{2}{3}$ volume; white blood-corpuscles, blood and hyaline casts. Decapsulation was performed and a small section of the kidney-tissue removed. The section showed moderate diffuse fibrosis and very abundant lipoid about the tubules. Decapsulation was followed by improvement for a short time, but later the course was entirely downhill until death

occurred. No autopsy was obtained. It is interesting to note in this case the fall in lipoids as the blood-urea increases.

Case 11.—Male. Diagnosis: Chronic parenchymatous nephritis. Admitted to the hospital with a history of recurrent ascites, vomiting and diarrhœa. On admission there was moderate œdema of the ankles and œdema of the optic dics. Urine: Sp. gr. 1035, acid; albumen $\frac{2}{10}$ volume; white and red blood-corpuscles, hyaline and granular casts. During stay in hospital the appendix was removed because of an acute attack of appendicitis. Improved and sent to convalescent home.

Case 12.—Female, aged 32. Diagnosis: Chronic parenchymatous nephritis. History of frequent headaches for five years. History of scanty urine, puffiness of eyes and swelling of legs for past three months. On admission there was massive œdema of lower extremities. Blood-pressure, 160 systolic, 120 diastolic. Urine: Sp. gr. 1008–1015; albumen $\frac{1}{8}$ to $\frac{1}{6}$ volume; granular and fatty casts. Southey tubes were inserted in lower extremities and drainage was copious. Some improvement.

Case 13.—Male, aged 15. Diagnosis: Acute nephritis. Headache, vomiting and hæmaturia of one day's duration. No œdema. Blood-pressure, 110 systolic, 70 diastolic. Urine scanty, acid; sp. gr. 1009–1020; albumen $\frac{1}{3}$ volume; red blood-corpuscles.

Case 14.—Male, aged 10. Diagnosis: Acute nephritis. Idiopathic epilepsy. History of headache, vomiting and puffiness of face of two weeks' duration. Epilepsy for several years. Urine: Sp. gr. 1010–1015; albumen $\frac{1}{20}$ volume; red blood-corpuscles. Discharged improved.

Case 15.—Female. Diagnosis: Albuminuria of pregnancy. History of headaches and œdema. On admission there was œdema of ankles, albuminuria and systolic blood-pressure 170. The blood-pressure rose to 215 systolic. Following therapeutic abortion, recovery was rapid and blood-pressure returned to normal. Urine: Sp. gr. 1014; albumen $\frac{1}{4}$ volume; no casts.

Case 16.—Young female. Diagnosis: Toxæmia of pregnancy. The patient was about six months pregnant. Five weeks' history of headache, drowsiness, nausea and vomiting. Blood-pressure at this time was 180 systolic. Unconsciousness supervened at intervals. Spontaneous abortion. On admission to hospital the patient was drowsy and blood-pressure 115 systolic. Albuminuric retinitis. Urine examination: Albumen $\frac{1}{4}$ volume, acid, pus; urine culture showed *Bacillus proteus*. Later marked improvement. Sent to convalescent home. Incomplete recovery; died September, 1925.

Case 17.—Middle-aged male. Diagnosis: Congenital cystic kidneys. Ten years ago a ruptured right cystic kidney was removed at the London Hospital. Recovery was complete. Ten months ago there occurred loss of appetite, nausea, vomiting, diarrhœa, headaches, cramps in the legs and loss of weight. The symptoms have all been progressive. On examination the patient showed drowsiness, slight œdema of ankles and a large mass in the left flank. Blood-pressure, 160 systolic, 120 diastolic. Urine: Sp. gr. 1018; trace of albumen. Death occurred one week after admission. The remaining kidney was only a mass of cysts. No normal tissue macroscopically.

Case 18.—Middle-aged female. Diagnosis: Urinary tract infection; uræmia. Admitted to the hospital nine months ago with fever, dizziness, vomiting and pain in the back. Urine showed albumen, blood and pus. Urine culture showed *B. coli* and *Staphylococcus albus*. Blood-pressure, 155 systolic, 110 diastolic. Discharged improved. Two weeks ago the original symptoms returned. Vomiting, oliguria, unconsciousness and incontinence were also noted. Blood-pressure, 145 systolic, 90 diastolic. No œdema. Heart and vessels normal. Urine: Sp. gr. 1018; albumen $\frac{1}{8}$ volume; acetone, bacteria, pus.

Case 19.—Middle-aged male. Diagnosis: Pyonephrosis. History of frequency followed by acute retention due to urethral stricture four years ago. Recently frequency, vomiting, diarrhœa, dysuria, nocturnal frequency, and specks before eyes. On admission he looked ill. No œdema. Pyrexia and tenderness in flanks. Urine: Alkaline; sp. gr. 1015; albumen $\frac{6}{10}$ volume, blood and pus. Gradually grew worse and died. At autopsy the kidneys were simply two sacs of pus.

Case 20.—Female. Diagnosis: urinary tract infection. History of frequency, pain in loins, fever and chilly sensations. On admission, pyrexia, tenderness in both loins, and slight increase in blood-pressure. Urine: Sp. gr. 1012: albumen $\frac{1}{8}$ volume, pus. Culture of urine showed *B. coli* and *B. proteus*.

Case 21.—Male, aged 60 years. Diagnosis: arterial hypertension (cardio-renal disease); heart failure. Nine months' history of weakness, dizziness, œdema of ankles and dyspnœa. On examination there was œdema of the ankles, enlarged heart, thickened arterial walls and blood-pressure 240 systolic, 150 diastolic. Urine: Acid sp. gr. 1006; trace of albumen; few granular casts.

Case 22.—Male, aged 60 years. Diagnosis: Arterial hypertension (cardio-renal disease). Palpitation, headache, dyspnœa, and œdema of the ankles for the past five months. Cardiac enlargement, thickened arterial walls, slight œdema of ankles. Blood-pressure, 210 systolic, 105 diastolic. Urine: Sp. gr. 1015–1045; albumen 0–$\frac{1}{10}$ of volume; granular casts.

Case 23.—Male. Diagnosis: Pulmonary tuberculosis. Renal tuberculosis (?). The patient gave a history of cough, weakness, loss of weight in recurrent attacks for the last fourteen years. Pleurisy three years ago. Œdema of ankles of one year's duration. Recently vomiting, cough and dyspnœa. Physical examination showed clubbed fingers, cachexia, and cavitation in right upper lobe. Blood-pressure, 100 systolic, 65 diastolic. Urine examination: Albumen $\frac{1}{4}$ volume; pus, *Bacillus coli*; no tubercle bacilli found, no casts. Tubercle bacilli were found in the sputum. During his stay in the hospital there was marked improvement, and he was sent to a convalescent home.

SUMMARY.

The results of the examination of the plasma-lipoids and plasma-proteins in eighteen cases of renal disease are reported.

The plasma-lipoids were increased in five out of seven cases of chronic parenchymatous nephritis examined.

In other forms of renal disorder examined no similar increase of plasma-lipoids was found. The average cholesterol and lecithin values were indeed less than those found in normal individuals. The average fatty acid value on the other hand slightly exceeded that found in the normals.

No quantitative relationship appeared to exist between the plasma-lipoids and the plasma-proteins.

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ON THE USE OF TRYPSINIZED CASEIN, BROM-THYMOL-BLUE, BROM-CRESOL-PURPLE, PHENOL-RED AND BRILLIANT-GREEN FOR BACTERIOLOGICAL NUTRIENT MEDIA.

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For the purpose of producing cheap culture media we began, four or five years ago, at this Institute, to experiment with trypsinized casein, basing our work on Cole and Onslow's (1916) communication. After various orientating experiments, which we shall omit to discuss, we have adopted the following mode of procedure, which in the daily routine work for about three years has proved to be fully satisfactory, and to possess several advantages beyond mere economy. The different ways in which we have applied this medium in connection with brom-thymol-blue and other dye-stuffs will also be accounted for.

1. *Preparation of the Basic Medium.*

We have employed a Danish casein (price 155 kroner per 100 kgm.). It is a dry, light yellow powder, which, according to a Kjeldahl analysis, courteously undertaken for us by Dr. E. Warburg, has a nitrogen content of 12·38 per cent. (after washing with a heated acetate mixture according to I. Bang's method it contains 11·75 per cent. N).

To 3 kgm. of casein add 30 litres tap-water and mix carefully. Stir continually while the mixture is heated to boiling-point, and then add enough of a strong sodium hydroxide solution to give a red colour with phenolphthalein corresponding to pH 8·5 to 9 when tested after being kept for about ten minutes at 90° to 100° C. A more accurate adjustment of the reaction has not been found necessary. The casein employed by us requires about 700 c.c. of 5 N. NaOH. The amount required for other casein preparations may, however, be different.

The casein will be dissolved by the addition of the sodium hydroxide, so as to form a semi-transparent, thick-flowing liquid. This is distributed into big flasks, and, after cooling to 37° C., 5 gm. of pancreatin "Rhenania" and 75 c.c. of chloroform are added for each kilogramme of casein (= about 10 litres of the liquid). The flasks should be closed with a cork stopper, thoroughly shaken and placed in the thermostat at 37° C., to be shaken daily for the first three or four days. During the first twenty-four hours the contents of the flasks will be diffusely turbid, to be gradually converted, however, into a perfectly clear liquid of a fairly intense yellow with a steadily decreasing precipitate. When this precipitate, at the end of about a week, reaches a level of about the middle of the flask, digestion may be discontinued.

Should digestion proceed too slowly, the cause may be that the reaction has become acid; to make quite sure upon this point it is advisable to check the reaction in the course of a few days, and, if necessary, adjust to pH 7·6 to 7·8 by adding NaOH. In our experience, however, it has not been necessary to control the reaction during the act of trypsinization.

At the end of trypsinization 200 c.c. of pure concentrated hydrochloric acid, previously diluted in about one litre of water, are added for each kilogramme of casein employed. This highly acid mixture is boiled for about five minutes and filtered through paper. We have boiled it in a tinned copper vessel. Of course it would be more safe to boil in glass flasks. Whether the medium would gain in any way by avoiding the contact with "metal" we have not examined. Quite recently we have in different ways compared a tryptic digest with and without boiling, which does not seem to make any essential difference. The filtrate is now measured off; if it amounts to less than 10 litres per kilogramme of casein, the deficiency is made up by adding tap-water. This "acid stock solution" should be stored in the cooling-room. If more than a month elapses before it is used, add ½ per cent. chloroform to prevent development of moulds.

Two samples of casein filtrate, of which the one was some months old, the other one quite freshly made, were tested for dry substance and amino-nitrogen:

	I.	II.
Dry substance . . .	8 per cent.	8·3 per cent.
Amino-N	0·28 „	0·295 „

The value for amino-N is determined by formol titration as follows: adjust to the neutral point of highly sensitive azolitmin paper (pH about 6·8), add surplus of formalin + phenolphthalein, which has previously been alkalinized to a weak red colour. Then add N/10 NaOH until a red colour corresponding to a pH of about 9 is obtained. 1 c.c. of N/10 NaOH equals 1·4 mg. of amino-N.

When a portion of the acid stock solution is to be used it is brought to pH 7·7 with NaOH (quantity determined after ten minutes' boiling, cooling and diluting with tap-water in a proportion of 1 to 2 or 5). When other portions are taken at subsequent times the titrations need not be repeated, but the previously calculated quantity of NaOH may be added, if the same NaOH solution and measuring vessels are used. If chloroform has been added this is driven out by boiling for one to two minutes. This "alkaline stock solution" is now immediately further prepared into "indol-bouillon" or into "Drigalski-plates"; it has not been necessary to filter after the addition of NaOH.

2. "*Indol-bouillon.*"

As emphasized by Cole and Onslow and others, trypsinized casein is especially adaptable as a medium for testing indol formation.

One part alkaline stock solution and 2 parts tap-water are autoclaved and distributed with sterile precautions into test-tubes. We make an extensive use of this medium in the routine investigations for pathogenic intestinal bacteria, since the examination can be discontinued immediately when a

bacterium is seen to form indol, provided that the investigation concerns only the usually occurring bacteria of the typhoid and paratyphoid groups pathogenic to man, or the Shiga dysentery bacilli.

We tested a number of strains of typhoid, paratyphoid and Gärtner bacilli, not only those isolated by ourselves, but also a number of strains from other laboratories; all the strains gave negative indol reactions. As a rule, the indol-forming strains give a vigorous reaction in a culture of not more than twenty-four hours.

In order to show that it is quite safe to perform indol reaction tests at the end of twenty-four hours, we prepared cultures from 84 casually chosen colonies from fæces plates; we preferred colonies which did not resemble typical *B. coli*, and among these some which did not ferment lactose. From each colony two test-tubes of " indol-bouillon " were inoculated. One of these cultures was tested at the end of twenty-four hours by adding Ehrlich's indol reagent (paradimethylamidobenzaldehyde, 4 gm.; 96 per cent. alcohol, 380 c.c.; concentrated hydrochloric acid, 80 c.c.), so as to form a layer on top. A positive reaction is indicated by the appearance of a red disc at the junction of the reagent and the medium to be tested (Norton and Sawyer (1921) and others). The second culture was tested at the end of seven days. To the tubes which gave a negative reaction in the latter test potassium persulphate was added and they were shaken.

Of these 84 strains, 75 gave a vigorous indol reaction both after twenty-four hours and after seven days, while the remaining 9 strains did not show any trace of reaction at the end of any of these periods, and neither after addition of potassium persulphate. This seems to indicate that twenty-four hours' incubation is sufficient for cultures on trypsinized casein when it is a question of deciding whether one of the common Gram-negative rods from fæces is an indol-former or not. However, this holds good only of strains which have a lively growth; for, when testing a series of Flexner dysentery strains, we observed a few cases in which the indol-reaction was negative after twenty-four hours and positive at the end of seven days. As some authors have recorded that bacteria may form indol, but utilize it again in their metabolism, it is presumably advisable always to make the test after twenty-four hours, and, if the result be negative, it may be repeated with a new culture at the end of a longer period.

The experiments described here, as well as the experiences we have otherwise made, seem to indicate that the method of pouring the yellow indol-reagent (without potassium persulphate) on the top of the culture is just as reliable as the method devised by Böhme, *i. e.* mixing one portion of culture with half a portion of Ehrlich's indol reagent and half a portion of a saturated solution of potassium persulphate. The technique of the former method is simpler, which is essential in routine laboratory work, and the reaction occurs almost instantaneously.

This " indol-bouillon " (in a higher dilution) is also applicable for fermentation tests in fluid medium, but meat-extract-pepton-bouillon yields more distinct reactions, and as the reduction of the expense within this field would only be slight, the latter medium should no doubt be preferred.

By addition of 0·1 per cent. glucose to the " indol-bouillon " bacterial

growth will be considerably promoted, so that the culture develops at the same rate and obtains the same density as when grown in meat-bouillon.

Cultures of typhoid, paratyphoid B, dysentery and cholera bacilli in "indol-bouillon" (in a dilution as 20 litres from 1 kgm. casein) were tested for agglutination with their specific immune sera. All the tests showed normal agglutination. In one case a paratyphoid culture was tested in "indol-bouillon" (30 litres from 1 kgm. casein) both with and without glucose. The agglutination reaction took place at a more rapid rate and was more complete in the medium which contained glucose.

Typhoid, paratyphoid B, cholera and *proteus* bacilli showed normal motility in fluid casein media.

For the more fastidious bacteria, such as meningococci, Pfeiffer bacilli and others, we did not find the medium suitable even though ascites, hæmoglobin solution or the like had been added.

3. *Modified Drigalski-Plates.*

One part alkaline basic solution is diluted with 5 parts tap-water. From this a 2½ per cent. agar is prepared in the usual way; it is sterilized in the autoclave. One litre of this "casein agar" is melted, and to this is added a solution of 10 gm. lactose, 10 gm. saccharose* and 1 gm. sodium thiosulphate in 40 c.c. of a 0·2 per cent. aqueous solution of brom-thymol-blue. The solution is boiled for five minutes before being added to the agar. Furthermore, 5 c.c. of a 0·1 per cent. solution of crystal-violet are added. When poured into plates, this mixture should be of a pure blue colour. If greenish a proper quantity of NaOH should be added, as otherwise the change of colour will not be as distinct as desirable. On the other hand, it is important that the medium should not be more alkaline than necessary, as the pathogenic bacteria might then be inhibited in their growth.

Since K. A. Jacobson (1910) showed that the growth-inhibiting action of repeated autoclaving in a definite typhoid strain could be eliminated by adding sodium thiosulphate (among other substances), we have used this substance for our Drigalski-plates.

We use only half the usual volume of crystal violet. This has proved sufficient to inhibit the growth of cocci and Gram-positive rods to such a degree that, either they do not grow at all, or, if they grow, they form only quite small colonies, while typhoid, paratyphoid and dysentery bacilli are but slightly checked in their growth. It is perhaps even an advantage that the cocci are not completely checked; for, in testing for pure cultures, if we spread, for instance, typhoid colonies in streaks on our medium, contamination with cocci will as a rule distinctly manifest itself by growth of colonies inside the typhoid streak.

For the aqueous solution of brom-thymol-blue, dissolve 1 gm. brom-thymol-blue in 500 c.c. N/200 NaOH (475 c.c. distilled water + 25 c.c. N/10 NaOH). The flask is placed in the thermostat at 37° C., or, better, at 55° C., to be frequently shaken until the substance is completely dissolved.

This substitution of brom-thymol-blue for the usually employed litmus or

* We have employed a common domestic sugar with perfectly satisfactory results.

azolitmin, which we used as suggested by Kuczynski and Ferner (1923), proved to be of essential advantage as the change of colour is far more distinct. In fermentation tests for bacteria in fluid media we also use brom-thymol-blue as indicator, but in a concentration of 1 : 40,000, *i. e.* 12 c.c. of a 0·2 per cent. solution per litre (Baker, 1922), by which the disturbing change in the indicator caused by reduction is practically totally eliminated (more so than with brom-cresol-purple).

Previously, before using trypsinized casein for our Drigalski-plates, the nutrient basis was a "bouillon" made of 1 per cent. Liebig's meat extract, 1 per cent. Witte's pepton and ½ per cent. NaCl. This bouillon is still used for our fermentation tests in fluid media.

In various ways we have made quite certain that "Drigalski-plates" made from trypsinized casein yield as good and reliable growth of the pathogenic intestinal bacteria as plates prepared from meat extract and pepton. In the first place, colonies of typhoid and paratyphoid bacilli, when appearing at all, were constantly seen to grow to a similar extent on the casein plates as we were accustomed to see on the media previously used. Besides, we examined 82 samples of fæces by plating on two sets of "Drigalski-plates," prepared each from one of the basic media but otherwise identical. Pathogenic bacteria were found in 16 of the samples; the distribution of the positive findings was as follows:

	Positive on both media.	Positive on meat-extract-pepton only.	Positive on the casein medium only.
Typhoid	3	1	1
Paratyphoid B	7	0	3
A dysentery-like bacillus	1	0	0

And, finally, a pure culture of typhoid bacilli (recently isolated) and a pure culture of Shiga-dysentery bacilli were plated on the following four media:

I. Common bouillon-pepton-agar (from horse's meat).
II. "Drigalski-plates" prepared from meat-extract-pepton-agar, pH = 7·7.
III. „ „ „ trypsinized casein, pH = 7·7.
IV. „ „ „ „ „ pH = 7·2.

On two plates of each medium was spread a drop of properly diluted culture suspension of each of the two bacterial species, and the colonies were counted at the end of twenty-two to twenty-four hours. Besides the number, the size of the more isolated colonies is stated in the table below:

	Typhoid culture.	Dysentery culture.
I.	1051 : 1370 : 3–4 mm.	525 : 633 : 3 mm.
II.	1090 : 1156 : 1¼ „	661 : 622 : 2 „
III.	1376 : 1301 : 1¼ „	635 : — : 1 „
IV.	1308 : 1278 : 1½ „	641 : 562 : 1¼ „

It appears from this series of tests that the bacteria developed equally well on the casein-agar as on the other media. Furthermore, it is seen that the size of the colonies was slightly less with a pH of 7·7, than with the more optimal reaction of pH 7·2, though they were not diminished in number.

While the typhoid colonies were equally large on II and III, the growth of the dysentery bacilli was more marked on II. Experience shows also in

other respects that casein media are less suitable for bacteria of the dysentery-group. It is true that most of the strains of dysentery bacilli we examined grew well enough on these media; but two definite strains of Flexner dysentery bacilli (old laboratory strains), by repeated tests, grew only quite negligibly, or not at all, on casein media, while they grew well on meat-extract pepton-agar and excellently on bouillon-pepton-agar. Therefore in cases suspicious of dysentery, we supplement our usual examination with inoculation on a plate of bouillon-pepton-agar to which is added 1 per cent. lactose, and using brom-cresol-purple as an indicator, but omitting saccharose, crystal violet and sodium thiosulphate. We chose another indicator partly to be able to distinguish the two kinds of plates from each other, and partly in view of the possibility that the natural sugar content of the bouillon might cause weak acid reactions, which would produce changes in colour with brom-thymol-blue but not with brom-cresol-purple, since the reaction range of the latter indicator lies farther to the acid side. The colour reaction is, however, not so distinct as with casein-agar-plates with brom-thymol-blue as indicator. Why we omitted saccharose and crystal violet need hardly be explained.

We shall just point out that in cases in which we isolated bacteria of the dysentery-group from fæces, with a single exception these developed quite well on the casein-plates, and during the first twenty-four hours the colonies always retained a pure blue colour.

Our medium possesses two or three special advantages as compared with corresponding plates prepared from meat-extract or bouillon. In the first place, *B. proteus* is inhibited to such a degree in its spreading along the surface that it is very exceptional to find a plate quite overgrown with these micro-organisms. In the second place, the blue and yellow colours appear beautifully pure and clear owing to the basic medium being almost colourless. Furthermore, the differences between *B. coli* and *B. aërogenes* seem to be more conspicuous on this medium than on most others.

In order to study the significance of a shorter or longer period of digestion for the casein we prepared plates from casein which had undergone digestion for one, two, four or eight days. The filtrates contained 0·18, 0·21, 0·24 and 0·28 per cent. amido-nitrogen respectively. Each filtrate was diluted as usual (1 + 5), agar was added and it was sterilized in the autoclave. On each of the four media cultures of the following bacteria were made: two dysentery-strains grew well on all four media, best, however, on the one which had been allowed to digest for the longest period; typhoid-strains grew almost equally well on the last three media, while their growth was slightly less vigorous on the medium which had only digested for twenty-four hours. Paratyphoid A and B grew almost equally well on all four media.

In tests with "indol-bouillon," which had partly been subjected to the usual period of digestion, and partly been allowed a much more intensified digestion, both typhoid, paratyphoid B, Aertrycke and coli bacteria grew best in the latter medium.

Collating all our experiences, we may conclude that the growth of the pathogenic intestinal bacteria is, to a comparatively slight degree only, influenced by variations in the degree of digestion. We should rather prefer to have the digestion intensified if it were not that regard must also be paid to

the amount of pancreatin consumed, and to having the process finished within a reasonable period.

4. *Comparison of Indicators.*

Although the colour reactions obtained by using brom-thymol-blue as an indicator are so sharp that one would not expect to find other indicators superior, we undertook some comparison tests with a selection of the best known media for fæces investigations, *i.e.* endo-agar; acid fuchsin ("Andrade") agar; China-blue agar (according to Bitter, 1911), without malachite green, however; Congo-red agar (Liebermann and Acél, 1914); alizarin-agar (Guth, 1909); eosin-methylene-blue-agar (Holt-Harris and Teague, 1916); and water-blue metachromic-yellow-agar (Gassner, 1918). As it was our aim to compare the different indicators we used the same basic medium throughout, *i. e.* trypsinized casein to which were added 1 per cent. lactose and 1 per cent. saccharose, while otherwise we followed the descriptions given by the various authors. Cultures were made by inoculation partly from fæces samples, partly from pure cultures of coli, aërogenes and pathogenic intestinal bacteria. It soon became evident that none of these media yielded as reliable and as sharp reactions as the plates with brom-thymol-blue. And besides, on the media which did not contain crystal violet or similar growth-inhibiting substances, non-desirable colonies developed to a far greater extent than on the other plates. Of course, one cannot pronounce any decisive judgment on the various media without having prepared them according to the authors' instructions throughout; but if the other indicators, in contra-distinction to brom-thymol-blue, require a specific constitution of the basic medium in order to exert their full effect, this must at any rate be considered a disadvantage. Moreover, it is essential to know what process the colour-reaction is an expression of. Using brom-thymol-blue, one may feel quite sure that a yellow colouring in and around a colony proves that the culture concerned has split up lactose or saccharose in forming acid, while in several of the other cases the relations are not quite so clear.

It has been claimed an advantage of eosin-methylene-blue agar and water-blue-metachromic-yellow agar that the colour-reaction takes place within the colony itself, and does not at all, or to a slight degree only, spread to the surrounding areas. This is not to be denied; but, using an adequate technique in inoculating the plates, one may obtain plates with several hundred colonies which lie so detached that the diffusion-zones do not impede the estimation of each individual colony; and again, it is much easier and the results become much more reliable if transfers are made from well-isolated colonies than from very crowded plates, so that the disadvantage of acid diffusion is in reality of minor importance. It is provided, of course, that examination of the plates is undertaken not later than eighteen hours after inoculation.

5. *Brilliant- and Malachite-Green Agar.*

Trypsinized casein agar has also proved applicable as a basic medium for malachite-green and brilliant-green plates. We have employed the same agar as that of "Drigalski-plates," but adjusted it to a weak acidity (pH 6·6 to 6·8)

by adding a proper quantity of autoclaved lactic acid solution (15 c.c. N/2 lactic acid per litre). Malachite-green and brilliant-green are then added in concentrations which cannot be fixed once for all, as they may depend on variations in the agar, which are not definitely known. At present we use 30 c.c. of a ½ per cent. solution of brilliant-green per litre agar. It has been claimed that brilliant-green is more elective than malachite-green; presumably, however, the fact is only that brilliant-green exerts a stronger action in a given concentration without there being any qualitative difference in the action of the two substances. We have plated typhoid, paratyphoid, Gärtner bacilli and various coli bacilli on brilliant-green and malachite-green agar in a series of varying concentrations; the results showed that when applying the two dye-stuffs in concentrations of identical colour intensity, they were also identical in their action on various bacterial cultures. Only one brilliant-green and one malachite-green preparation were examined.

A pronounced elective action of malachite-green and brilliant-green was seen only with paratyphoid B and Aertrycke bacilli, and, to a somewhat slighter degree, with Gärtner bacilli. In the case of typhoid we tried, among other things, brilliant-green in the form of bouillon (horse-meat-peptone bouillon adjusted to pH 6·8, and the brilliant-green in such concentrations as 1 : 60,000 and 1 : 120,000), as recommended by Killian (1924) for cultivation from urine. No elective action could be observed in cultures from urine samples from about 20 cases of typhoid fever; 3 cases showed growth of typhoid bacilli in pure culture, by direct inoculation on Drigalski-plates as well as in both the brilliant-green bouillons; in the remainder of the cases no typhoid bacilli were found by any of the methods.

Corresponding tests with mixed typhoid and colon bacilli did not give any positive results either. In the case of paratyphoid B, however, the brilliant-green bouillon had a very pronounced elective effect.

Whether the favourable results arrived at by Killian and other authors in typhoid tests are due to the malachite green or brilliant-green preparations employed, or to other factors (for instance in regard to the preparation of the medium), we cannot decide.

After having used malachite green and brilliant-green plates without any special additions for a lengthened period we tried whether addition of lactose, saccharose and an adequate indicator would facilitate examination of the plates. As indicator we used, first, brom-cresol-purple without any satisfactory results. Next we tried phenol-red: 10 gm. lactose and 10 gm. saccharose are boiled in a 0·2 per cent. solution of phenol-red (1 gm. phenol-red dissolved in 460 c.c. distilled water + 40 c.c. N/10 NaOH) and added to the melted agar. As the reaction-point of the non-inoculated plates lies at the acid end of the reaction-range of phenol-red, the paratyphoid colonies, which are strongly alkaline-producing, will show up red, as the brilliant-green decolorizes simultaneously while the phenol-red reacts by changing into red and purple. Colon and aërogenes bacilli intensify the green colour without producing any qualitative change. The result is therefore a beautiful contrast effect, which is, moreover, peculiar in that it is here the pathogenic bacteria which produce the change in colour, while otherwise it has primarily been the colon-bacilli which changed the colour of the media.

As a rule the red colour appeared distinctly at the end of eighteen hours; it is, however, advisable to allow the plates to stand in the thermostat for twenty-four hours.

We have used the medium for about one year and have found it very useful. One might fear that addition of lactose and saccharose to brilliant-green agar would promote the growth of colon bacilli so as to make the medium less elective; however, this does not seem to be so, and, as is known, an addition like the above described has also been used in several other laboratories in connection with an indicator ("Andrade," and others).

On the whole there are scarcely any of the details in this work which have not been previously mentioned, somehow or other, by other authors; yet we thought that the way in which we have selected and combined some of the results of recent years' investigations within this field of bacteriology might be of some interest to others.

SUMMARY.

1. Trypsinized casein is well adapted for examination for pathogenic bacteria of the typhoid and paratyphoid group; it possesses the following advantages:

(*a*) It is an excellent medium for indol tests.

(*b*) It is very cheap.

(*c*) "Drigalski-plates" prepared from this medium, as described in this paper, yield very beautiful and distinct colour reactions, and possess a marked inhibitive action on the growth of *B. proteus*.

2. Brom-thymol-blue seems to be the best indicator both with Drigalski-plates and for fermentation tests in fluid media.

3. Phenol-red is especially valuable as an indicator in connection with brilliant-green.

We must not conclude this communication without thanking Miss Ingeborg Bjerre, pharmaceutical chemist, for her assiduity and care in preparing the various media.

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GLYCOLYTIC ACTIVATION BY TUMOUR EXTRACTS.

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In numerous kinds of experiments tumour extracts are made use of, *e.g.* for the purposes of immunization, for demonstration of protective power towards tumour-cells, and also recently in the work of Gye (1925), where different tumour extracts serve as "specific factor," in the presence of which only a tumour infection is possible. Though there must undoubtedly exist a multitude of active principles in a tumour extract, we can only expect to make any progress in our knowledge when we know more *details* about the chemical and physico-chemical properties of these extracts. It seems desirable to divide this multitude of active principles into definite functions and to study each separately.

In this paper I will discuss the bearing of tumour extracts upon glycolysis in normal and malignant tissues. This study seems to be a logical extension of the work of Warburg (Warburg and collaborators, 1923, 1924, 1925) and of my own.

I consider, as generally known from the investigations of Otto Warburg, that:

(1) Glycolysis of the tumour-cell is remarkably high:

(2) That the strong glycolytic action is maintained by the tumour-cell even under aërobic conditions, in opposition to the behaviour of normal and embryonic cells, which are able to consume all of the lactic acid formed when under aërobic conditions.

In a paper published in 1924 (Waterman, 1924), after verifying the remarkable results of Warburg also in human tumours, I studied the effects of the addition of various cell-free tumour extracts on the glycolytic properties of normal cells. In the present paper I will deal further with this subject.

TECHNIQUE.

A. *Choice of Normal Tissues.*

The most suitable tissue of our small laboratory animals appeared to be the kidney. It is one of the largest parenchymata, is not particularly connected with carbohydrate regulation, and shows, even under anaërobic conditions, no very important production of lactic acid.

According to the method indicated by Warburg, the tissue was cut into thin slices with a razor.

B. *Determination of Acid Formed.*

In my earlier work, mentioned already, I applied the manometrical technique of Warburg, where the quantity of lactic acid formed is measured indirectly by

the quantity of carbonic acid driven out from the bicarbonate of the Ringer solution. In this work glycolysis was observed under atmospheric oxygen-tension.

Later, Warburg carried his distinction still further between aërobic and anaërobic conditions, by examining in oxygen or in nitrogen-atmosphere.

Owing to various circumstances I could not make use of this manometrical method, and was obliged to adopt a more simple method, viz. that of direct acid-titration, which, however, proved useful and sensitive enough. After many control experiments, I think I am entitled to trust this simple titration technique, the more as Warburg shows in his recent publications that, practically, no other acid than lactic acid is formed under the conditions of these experiments, and that, indeed, the quantity of lactic acid formed can be determined simply by two bicarbonate dosages.

Further, as with the manometrical technique, the quantity of acid produced corresponds very well with the quantity of sugar which has disappeared, as is illustrated by Table I (at the end of this paper).

We see from that table that 80 to 90 per cent. of the sugar decomposed is represented in the quantity of acid formed. Nevertheless a small quantity of the decomposed glucose must be present in another form than that of lactic acid.

c. *Acid-titration.*

The thin slices of kidney (rats and mice) are thoroughly mixed on a watch-glass, divided into smaller particles by mincing superficially with scissors and weighed out into equal portions (100–150 mgm.). The tissue is brought into 50 c.c. Erlenmeyer flasks; 3 c.c. of a Ringer solution is added of the following composition:

NaCl	8·5 per cent.
KCl	0·02 „
$CaCl_2$	0·02 „
Bicarbonate	0·02–0·025 per cent.
Glucose (Kahlbaum) . . .	0·15 per cent.
pH	= 7·5–7·6.

The particles of tissue are immersed in the fluid, and after closing with corks, the flasks are put into the incubator at 37° for two hours. No antiseptic is added.

It is evident from this description that the tissue treated in this way is under partly anaërobic conditions, and depends for its oxygen consumption only on the small quantity of oxygen dissolved in the Ringer solution at 37°. In examining the conditions, however, which govern glycolysis of normal tissues, some anaërobiosis must be maintained. This degree of anaërobiosis is the same in all experiments.

Further, for the purpose of testing the influence of tumour extracts and other chemical substances, under *pure* aërobic and anaërobic conditions, in other series of experiments the flasks were kept in atmospheres of pure oxygen and nitrogen. They were put into exsiccators, the air in which had been driven out and replaced by oxygen and nitrogen. In some experiments the atmosphere was freed from carbonic acid by a strong solution of KOH.

After two hours in the thermostat, 10 c.c. of absolute alcohol was added to the contents of each of the flasks. By filtering through ash-free paper a clear albumen-free filtrate was obtained.

This technique resembles very closely the method indicated by Rohónyi in determining blood alkalinity. As an indicator alizarin-sulfonic acid is added (3 drops of a 0·5 per cent. solution). This indicator has a range from pH 6·8–5·5.

By adding 1/200 N.HCl solution from a burette to 10 c.c. of the alcoholic solution, it is possible to change the colour from violet to yellow (pH 5·5), and, compared with controls where no acid-formation has taken place, the amount of acid needed for the complete colour-change is in inverse ratio to the quantity of acid formed. We may not, however, consider as quantity of acid formed, expressed in 1/200 N, simply the difference in titration value between the Ringer solution, diluted with alcohol in the same way, and the contents of the flasks. For this would be true only on the assumption that there were no buffer-substances in the titrated fluids. There are such, however. First there might be amino-acids present in the filtrate; further, when there is lactic acid formed in a bicarbonate solution, a buffer-system of Na-lactate and lactic acid will be formed. As we are working in alcoholic solution, it is not possible to calculate the buffer value of this system from the dissociation constants of lactic acid and indicator. Therefore a special set of experiments must be performed to determine this buffer-value.

Definite quantities of Na-lactate, corresponding approximately to the amounts of lactic acid formed out of the glucose, as known from determination by the Hagedorn-Jensen method, were titrated in alcoholic solution with the same indicator. The quantity of 1/200 N.HCl necessary to produce the yellow colour (pH 5·5) was noted. It was found that, as a rule, 0·2 c.c. was needed.

Therefore, in the determination of the real quantity of lactic acid formed, this value must be taken into consideration, and the original value augmented by 0·2 c.c. 1/200 N.

There is generally a fair agreement between the pH of the alcoholic filtrates judged from their colours and the values obtained by titration.

(D) *Preparation of Extracts.*

Tumours of most different origin were used. They were minced and thoroughly rubbed with quartz-sand, and diluted 1/10–1/20 with physiological salt solution. For dilution no use was generally made of Ringer solution, because in this medium the tumour extracts were slightly more alkaline than the original Ringer fluid. This would have caused complications, as glycolysis increases as a rule with increasing pH.

After standing from two to twelve hours in the ice-box, the extracts were centrifuged. The supernatant fluid was used (*a*) directly; (*b*) after filtration through Berkefeld N or Maassen candles (for Maassen filtration often a dilution in higher degree was required); (*c*) after filtration through asbestos-paper filter ("E. K. Schichte," Seitz-Werke, Kreuznach, Germany). Of course in (*a*) the fluid was turbid, in (*b*) and (*c*) clear.

Extracts were obtained in this way from human carcinoma, mouse carcinoma (No. 63) and sarcoma (tar), from embryos of mice and rats and from some normal organs (muscle, liver, spleen, pancreas).

RESULTS.

Though the results may be easily read from the tables, I think it inadvisable to intersperse the text with them, but to collect them at the end of this paper.

1. *Glycolysis.*

The glycolysis of normal mouse and rat kidney, in the semi-anaërobic conditions above described, lies between narrow limits. Though there are individual differences, especially in mice, the acid-formation by 100 mgm. of kidney amounts as a rule to 0·65 c.c. 1/200 N acid in two hours. With the buffer correction discussed above, and on the assumption that all the acid formed is lactic acid, we may say that per 100 mgm. of fresh tissue hourly $\frac{0{\cdot}65 + 0{\cdot}20}{2} \times \frac{90^*}{200} = 0{\cdot}19$ mgm. lactic acid is formed. Reducing the weight of fresh tissue to dry tissue by dividing by 5, then the quantity of lactic acid formed hourly by 1 mgm. of dry tissue becomes 0·0095 mgm. In rapidly growing malignant tissues this quantity amounts to 10 per cent. of the dry weight, or, per mgm., 0·1 mgm. As already shown by Warburg, the lactic acid formation by normal tissues is diminished notably when under aërobic conditions, without ceasing completely however.

2. *Physico-Chemical Conditions.*

As already pointed out by Warburg, the glycolytic process is influenced by the pH and bicarbonate content of the surrounding fluid. The acid formation proceeds parallel to increasing bicarbonate concentration. This statement harmonizes with the effect of the Ca″ ion. I found, and have described in a previous paper, that the acid production is reduced in a remarkable way by addition of calcium. This was shown by the manometrical as well as by the chemical method (determination by the Clausen technique); the method of acid-titration gave the same result.

3. *Acid-Formation from Sugar other than that added to the Ringer.*

There is also a slight acid formation in the Ringer solution without sugar. The only possible source is the trace of sugar or glycogen present in the tissue; the Hagedorn-Jensen titration shows that an amount of ± 0·03 per cent. glucose must be present.

4. *Influence of Extracts.*

The tumour and other extracts prepared in the way already described were added to the Ringer solution in a quantity varying from 0·2–0·3 c.c.

* The molecular weight of lactic acid being 90.

The total amount of fluid remained the same, viz. 3 c.c. As a rule the extract was brought first into contact with the tissue, and the complementary Ringer solution was added afterwards.

Addition of the extract alone, in this quantity, changed neither pH nor alkalinity to an appreciable degree. There was no trace of acid-production by the extracts alone, so that the acid formed could be explained only by the activity of the tissue. This control experiment proved at the same time that the lactic acid production could have nothing to do with bacterial activity.

Many dozens of extracts of different origin were tested. *They all agreed in so far that their addition to the glycolysing system increased the acid production in a remarkable way* (10–50 *per cent.*). This result, the outstanding fact of this examination, was already mentioned in my paper of 1924, but has now been fully confirmed. Particulars may be obtained in the tables.

The manifest activity of the extracts seems to depend also on the spontaneous glycolytic power of the kidney-tissue tested. There are individual differences in kidneys of mice in that respect. In rats results are more constant.

The only possible conclusion we can draw at present is that the tumour extracts contain an *activator*, which may be separated easily from the tumour-cell, and which accelerates the ferment action of the kidney tissue tested.

5. *Properties of the Activator.*

I have not been able to collect sufficient information about the nature of the activator.

The extract is not damaged in its activity by heating fifteen minutes at 65° C. It can be kept some days in the ice-box without special care as to anaërobiosis. Filtering through a candle lessened the activity, without destroying it. The substance is not injured by ether addition. Special experiments about the effect of ether addition will be related later.

Addition of substances such as succinates, which play an important part as hydrogen acceptors in normal carbohydrate metabolism, did not produce the same effects, nor did addition of unsaturated fatty acids.

Probably the activator acts in the first stages of sugar utilization, and is to be identified with the substance which catalyses both the first stages of oxydation and of fermentation. Such substances are known as pnein (Batelli and Stern), and more generally as muscle- and yeast-activators (Meyerhof, 1924).

This view harmonizes with the fact that insulin also is able to increase glycolysis of kidney tissue in a striking way. Formerly, experiments in that direction had yielded me negative results; but now, with the use of "Kahlbaum" dry insulin, in a quantity of three units or even less, and *without antiseptic*, a manifest activation took place. Without assuming in the least an identity between insulin and the activator in tumour extract, it should be remembered that in normal tissues also insulin-like substances occur, which may lower blood-sugar concentration to a certain degree, and which may possess identical prostetic groups in their molecule.

It seems impossible at the moment to approach the problem from its purely chemical side.

6. *Influence of O and N Atmosphere.*

As pointed out already by Warburg, the glycolysis of normal tissues is counter-balanced by oxidation in O atmosphere, in contradiction to conditions in N atmosphere. This observation was confirmed under the conditions of my experiments. *Further, the activating influence of tumour extract proved to be greater in anaërobic conditions (N atmosphere).* The same was the case with insulin.

7. *Effects of Washing the Tissue and Restoration Experiments.*

As is to be expected from the assumed nature of the activator, the slight spontaneous glycolysis of the kidney tissue ceases completely after washing the slices carefully with Ringer solution. For that purpose it is sufficient to wash 100 mgm. of tissue twice with 2 c.c. Ringer, simply by centrifugation.

In this way the tissue is not damaged at all in its vital properties, for it is now possible *to restore its glycolytic property by adding a small quantity of tumour extract.*

The only possible explanation seems that on the surface also of normal tissue, there is present a small quantity of "activator," which is easily separable from the cells, and in the absence of which all glycolysis ceases.

If now tumour tissue be subjected to the same washing, the glycolytic property is hardly, if at all, diminished. This proves that *tumour tissue produces or possesses the activator in a much larger quantity than normal tissue;* this is probably the explanation of all the phenomena observed.

The possibility of washing out the activator of normal tissue-fermentation is of interest for testing the activating properties of different substances and extracts. When the tissue is not washed, the real influence of the added substance is superposed on the spontaneous glycolysis, which varies with the individual. Therefore the testing of a washed preparation is the best method of examination as to activator properties of various substances.

As already mentioned, tumour extracts and filtrates restore the lost property of glycolysis. It is of great importance that normal serum of the same species has no restoring properties. The source from which the tumour extracts are obtained is of no importance. They are nearly all active. It is the same with insulin.

8. *Influence of Ether on the Activator.*

As already mentioned, when testing the lipoid-hypothesis as to the nature of the activator, some experiments regarding the sensitiveness for ether were performed. Definite quantities of tumour extract were shaken with ether in a separating funnel. After standing two hours the different portions were tested as to activity.

I distinguish three portions, viz. (1) the clear ethereal portion, (2) the watery portion, and (3) a turbid portion intermediate between both layers.

(1) The ethereal portion is inactive, and thus the activator seems not to be of simple lipoid nature, though this conclusion is not altogether warranted. It seems to me that it is generally not sufficiently borne in mind that addition of ether or other lipoid solvent (chloroform, phenol) might decompose a

substance of an originally lipoid nature The method of testing was simple: the ether was vaporized off, and the residue was dissolved in some c.c. of Ringer.

(2) The watery portion was active, but it appeared that the activity was greater in proportion to the quantity of the turbid intermediate layer which was allowed to mix with the clear watery portion.

(3) So it is probable that the active principle proper is present in the turbid intermediate layer, in absorption on the particles precipitated by the ether addition. These findings harmonize with the conception of the activator as an insulin-like substance.

9. *Control Experiments.*

(a) *Influence of serum.*—Spontaneous glycolysis also takes place in a milieu containing serum; the same technique of acid-determination is sufficient, and seems superior here to the manometrical technique which needs empirical gauging.

With normal serum I have not observed quantitative differences in glycolysis; it seems, however, that this is not the case with pathological sera (tumour sera and others). My studies in that direction have not been pushed far enough to warrant definite conclusions. It is obvious that such experiments might yield interesting results.

As already stated, addition of serum *cannot restore* the glycolytic property lost by washing; the serum does not contain an activator.

(b) *Extracts of normal tissues.*—It follows from the washing experiments that extracts of normal tissues must contain some activator. This conclusion is confirmed by experience. Extracts of muscle (chicken), spleen, liver, pancreas were tested. All of them showed a small but definite activity; they increased the glycolysis, and were able to restore the glycolytic function destroyed by washing.

Special attention was given to embryonic extracts; first, because glycolysis of embryo is remarkably high in anaërobic conditions; further, because embryonic tissue shows peculiarities in cultures "*in vitro*"; and chiefly, because embryonic extracts are so much used for purposes of cultivation and preparation of media.

Embryonic tissues possess noteworthy properties of activation; further, they are equally able to restore in a high degree the glycolytic activity lost by washing.

The extracts themselves do not show any glycolysis. For carrying out these experiments young embryos of mice and rats were used. They were got by Cæsarian section, rubbed with sterile sand in aseptic conditions, and emulsified in sterile salt solution.

DISCUSSION.

The general conclusion of this work must be that, in a multitude of different compounds, there must be a large quantity of glycolytic activator in tumour extracts.

The particular behaviour of the tumour-cell towards sugars is hereby explained, as a property not necessarily inherent to the cell itself, but separable

from it by extraction. The difference between a normal and a tumour-cell in that respect is only a quantitative one. A far larger quantity of activator exists in or on the tumour-cell, but it exists in all cells. In consequence of the greater amount of this activator of fermentation, this part of metabolism is increased (at least under our experimental conditions, where only katabolic processes can be observed), with a *relative* decrease of oxidation.

It is not at all improbable that under different circumstances the glycolytic activity of these extracts must be held as responsible for the phenomena observed. Thus it would become clear how in culture media, where anaërobic conditions must prevail, addition of extract would act as a stimulator of the "fermentative modus" of metabolism; it would be the same with *in vitro* cultures of tissue, where fresh extracts are indispensable, in so far as they improve the life-conditions of those cells which are very poorly supplied with oxygen.

Further, the analogy with the insulin-type points to the view that injection of tumour extracts, or spontaneous proliferation *in vivo*, may have an influence on the whole hormonic system; probably antagonistic substances must thus be produced in the organism, whereby this impetus in the direction of fermentation is counterbalanced. We might be inclined to speculate about the possibility of spontaneous augmentation of activator production in cells, by which they would obtain a tumour-character and, as in our experiments, normal kidney-cells are brought to fermentation. The problem is the more attractive, since, under definite experimental conditions, spontaneous generation of activator in ferment systems, transmissible in series, may be obtained, as was the case in the important work of Delezenne and Ledebt (1923) with pancreatic ferment. But our experiments above related are not of a wide enough scope to allow us to venture as far. It is equally impossible to say at this moment if glycolytic activity of the extracts has anything to do with other properties, such as are claimed by Freund and Kaminer (protection against lysis by normal serum), or by Gye (acting as specific factor).

CONCLUSIONS.

It is possible to increase the glycolytic activity of normal tissue by addition of tumour extract.

The activator substance present in tumour is not specific, as it is also contained in embryonic and to a lesser degree in normal tissue.

The different behaviour of the tumour-cell towards sugar utilization seems to depend only on quantitative differences, caused by the presence of a much larger amount of activator.

Regarding the nature of the activator, some properties seem to point to an analogy with an insulin-like substance.

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EXPLANATION OF THE TABLES.

All the values noted in the tables apply to volumes of 1/200 normal HCl solution, added by means of a microburette.

When there was some doubt as to the exact point of the end of titration both values are given, between which limits is the real end. The formation of acid is given by the difference in values between Ringer solution and the fluid where the different operations on the tissue were performed.

To simplify the tables it was necessary to omit some details, viz. first the values given by the control determinations, *which showed that no changes in the alkali contents were caused by the simple addition of extracts to the Ringer fluids.*

Further, the pH determinations, expressed in colours, varying from 6·8 to 5·5 are omitted, because the colour virages corresponded exactly to the titration values. Where law values are observed, the colours change from violet-red to yellow, in beautiful transitions. Should these experiments be repeated and controlled, I should strongly advise the observation also of the colour changes, which are very suggestive.

In the tables themselves the different operations are noted.

TABLE I.—*Relation between Quantities of Sugar Disappeared and Acid-Formed.*

	Acid-formed, expressed in 1/200 N.HCl.			Sugar disappeared (Hagedorn-Jensen).
Tissue: 100 mgm. kidney—	= 0·65 c.c.			
1. Corrected	= 0·85 c.c.	= 0·85 × 90*/200 mgm. lactic acid	= 0·382 mgm.	0·42 mgm.
2. ,,	= 0·62 c.c.	= ,, ,,	= 0·279 mgm.	0·39 mgm.
3. ,,	= 0·70 c.c.	= ,, ,,	= 0·32 mgm.	0·43 mgm.
4. ,,	= 0·73 c.c.	= ,, ,,	= 0·33 mgm.	0·41 mgm.

* The molar weight of lactic acid being 90.

TABLE III.—*Influence of Washing upon Glycolysis.*

Tissue.	Ringer solution.	Ringer glucose solution.	Tissue + Ringer glucose.	The same after washing.
Kidney of rat, 100 mgm.	1·07	1·08	0·58–0·62	1·15
	1·06	1·03	0·54–0·61	0·80–0·85
	1·09	1·16	0·71–0·77	1·16–1·20
	1·06	1·03	0·71	0·81–0·84
Scirrhus mammæ	0·90–0·95	0·91	0·66–0·73	0·73–0·79
Carcinoma 63, much necrosis, 100 mgm.	1·00	1·00	0·72–0·79	0·76–0·82

TABLE II.

Tissue.	Ringer solution.	Tissue + Ringer solution.	Tissue + Ringer glucose solution.	Tissue + Ringer glucose + extract.	Tissue + Ringer glucose + filtrate.	After addition of Ca.	Remarks.
1. Kidney of mouse, 100 mgm. in thin slices.	0·40	0·33	0·28	+ A 0·16	—	—	A = extract of human carcinoma-metastasis added = 0·2 c.c.
	+ A 0·38	—	—	+ A_1 0·19	—	—	A_1 = the same, heated for ½ hour at 70° C. and filtered.
	+ B 0·38	—	—	+ B 0·22	—	—	B = extract of mouse-carcinoma.
	+ A_1 0·40	—	—	+ B_1 0·16	—	—	B_1 = the same, heated at 70° C. for 15 minutes.
2. Kidney of mouse, 100 mgm.	0·32	0·23	0·19	0·14	—	+extract	Extract of mouse-carcinoma, with physiological salt solution added = 0·2 c.c.
	0·37	—	—	—	—	0·28	
						0·315	
3. Kidney of rat	0·45	0·32	0·20	—	—	0·385	
4. „ „	0·65	0·50	0·38	0·29	0·32	—	Extract and filtrate of a transplantable sarcoma.
5. Kidney of mouse	0·59	0·43	0·34	—	0·17	—	Sarcoma extract.
6. „ „	0·60	0·45	0·34	—	0·295	—	Carcinoma extract.
7. „ „	0·54	0·40	0·21	0·14 heated at 55°C. for 15 minutes. 0·14	—	—	Carcinoma extract.

TABLE IV.—*Restoration of Glycolysis by Tumour Extracts; Effect of Embryonic Extracts.*

Tissue.	Ringer.	Ringer glucose.	Tissue + Ringer glucose.	*Idem* after washing.	Addition of—
Kidney of rat	1·09	1·16	0·71–0·77	1·16–1·20	Tumour extract: 0·89–0·95
„ „	1·04	1·07	0·60–0·68	0·95–1·00	Tumour extract: 0·80–0·86
„ „	1·06	1·03	0·54–0·61	0·80–0·85	Serum: 0·98–1·03
„ „	1·05	1·08–1·10	0·54–0·63	0·79–0·83	Tumour extract: 0·66–0·70
„ „	1·00	1·00	0·54–0·58	0·95–1·00	Embryonic extract: 0·64–0·71
„ „	1·07	1·08	0·58–0·62	1·15	0·65–0·69

TABLE V.—*Influence of Ether upon the Activity of the Activator.*

Tissue.	Ringer.	Ringer glucose.	Tissue + Ringer glucose.	Tissue + Ringer + extracts.
Kidney of rat	0·88–0·92	0·93–0·96	0·42	+ 0·3 c.c. extract of tumour N 63, 0·39. *Idem* + 0·3 c.c. of same extract washed with ether, 0·32–0·34. + 0·3 ether extract, 0·59–0·63.
Washed kidney	„	„	0·64	+ 0·3 c.c. extract of N 63, 0·50. + 0·3 c.c. of same extract washed with ether, 0·37–0·40.
„	„	„	0·66–0·73	+ 0·3 c.c. ether extract, 0·70–0·77.
Kidney	0·89	0·83	0·37	+ 0·3 c.c. carcinoma extract N 63, 0·32. + 0·3 c.c. same extract after ether treatment, clear portion, 0·40–0·43. + 0·3 c.c. same extract, turbid intermediate portion, 0·30.
Washed kidney	„	„	0·68–0·72	+ 0·3 carcinoma extract N 63, 0·41–0·49. + 0·3 c.c. same extract after ether treatment, clear portion, 0·93–0·97. + 0·3 c.c. same extract (ether), turbid portion, 0·38–0·48. + 0·3 c.c. ether extract, 0·52–0·60.

TABLE VI.—*Influence of Extracts of Normal Tissues and Insulin.*

Tissue kidney.	Ringer.	Ringer glucose.	Tissue + Ringer glucose.	Tissue + Ringer glucose + extract of—
100 mgm.	0·67	0·74	0·49	+ 0·3 c.c. filtrate of muscle of chicken, opposite side to a Rous sarcoma, 0·56. + 0·3 c.c. of the Rous sarcoma of the same chicken, 0·39.
100 „	0·96	0·97	Washed tissue, 0·77–0·80	+ 0·25 c.c. liver extract 1/10, 0·61–0·67.
100 „	0·62	0·52	Washed tissue, 0·83	+ 0·2 c.c. liver extract 1/10, 0·65. + 0·2 c.c. pancreas extract, 0·67–0·73. + 0·2 c.c. spleen extract, 0·65.
100 „	0·62	0·52	Washed tissue, 0·83	+ 0·3 c.c. insulin, 0·09.
100 „	—	—	Fresh tissue, 0·23	+ extract of liver, 0·32. + „ spleen, 0·33. + „ pancreas, 0·44.
100 „	0·62	0·52	Fresh tissue, 0·23	+ 0·3 c.c. insulin, 0·13. The indicator is entirely turned to yellow.

TABLE VII.—*Influence of Oxygen Content of Atmosphere upon Acid-Formation.*

Tissue.	Ringer glucose.	Ringer glucose + insulin.	Tissue + Ringer glucose. O ∧ N	Tissue + Ringer glucose + insulin. 2 units (Kahlbaum). O ∧ N
Kidney of rat	1·02	0·96	0·59 0·39	0·43 0·27
„ „	1·01	0·89	0·79 0·58	0·48 0·34

Tissue.	Ringer glucose.	Ringer + tumour extract.	O ∧ N atmosphere.	Tumour extract. O ∧ N	Ringer + washed tissue. O ∧ N
Kidney of rat	1·09	0·98	0·61–0·55	0·52–0·36	0·89–0·74

O = atmosphere of pure oxygen; carbonic acid was eliminated by KOH.
N = anaërobical condition, in pure nitrogen atmosphere, or by immersing only in the Ringer's solution.

Insulin used was the product of Kahlbaum, which is delivered in little tabloids, and may therefore be dissolved in fluids without antiseptics. A tabloid weighs ±10 mgm. and is equal to 50 units. One tabloid was dissolved in 5 c.c. physiological salt solution, and 0·2–0·3 c.c. was made use of in the experiment.

ON THE EFFECT OF DEFICIENCY OF VITAMIN A ON THE BLOOD-PLATELET COUNT IN RATS.

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CRAMER, Drew and Mottram (1922) found a very considerable reduction in the platelet-count in rats suffering from a deficiency of vitamin A. This reduction amounted to over 100 per cent. in some of the animals examined. Bedson and Zilva (1923[1]), however, failed to confirm these findings, and were not able to observe any marked divergence between animals on a deficient diet and normals. The question of the technique of counting platelets formed the subject of a controversy between these workers, and in this connection it was found that, in the same rats, the number of platelets observed by Bedson and Zilva widely exceeded Cramer, Drew and Mottram's counts. This refers to the case of vitamin deficient rats. In normals a reasonably close approximation was observed, and it was suggested by the latter authors (1923) that the optical system used by Bedson and Zilva was responsible for the high counts, involving the inclusion of a large number of bodies not platelets, but described as lipoid globules, bacteria and protoplasmic *débris*.

This was denied by Bedson and Zilva (1923[2]), who carried out parallel counts with their own optical system and one similar to Cramer, Drew and Mottram's, with the result that no substantial differences were seen. A comparison was also made with two diluting solutions, Toison's fluid and 2 per cent. sodium citrate in saline, and these were found to be equally good. Bedson and Zilva, therefore, maintain their opinion that vitamin A deficiency is unattended by any significant reduction in the number of blood-platelets in the rat.

This question has been further investigated by the writer, and the following notes of the experiments carried out are now set forth. As reported elsewhere, the normal platelet count of rats in the author's colony averages slightly under 900,000.

Two sets of animals were investigated. In the first the rats were young, and averaged 80 gm. in weight at the time they were put on to the deficient diet, which had the following composition per cent. :

Purified caseinogen	22·1
Starch and cane-sugar	58·65
Olive oil (steam distilled)	15·0
Salt mixture	4·25

The salt mixture was based on McCollum's formula, modified by Hopkins, and its composition is reported elsewhere (Stammers, 1921).

Ten animals were used in the first experiment and the technique of

feeding, weighing, etc., has been previously described (Stammers, 1921). The animals were kept on the deficient diet for 90 days and, at the end of this period, the average weight was 104 gm. Keratomalacia was marked in 8 cases, so that a considerable degree of deficiency was assured. Details of the blood-count are as follows:

	1st day.	90th day.
Red-cells	9,290,000	9,270,000
Platelets	841,000	835,000

The technique of making the preparations and counting was precisely that described by Cramer, Drew and Mottram, except that the optical system used consisted of a Bausch & Lomb 1·9 mm. objective, with Abbe condenser and Zeis compensating ocular No. 5. In some cases parallel counts were made with a 4 mm. Zeiss apochromatic objective, and no significant differences were observed. It is noteworthy that in no case were any abnormal bodies seen during the counting.

In the second experiment adult animals were used. Six rats, average weight 180 gm., were placed on the same diet for 90 days. The average weight at the 90th day was 197 gm. One animal died during the progress of the experiment and four of the survivors developed keratomalacia. The same technique of counting was employed and the following figures were obtained:

	1st day.	90th day.
Red-cells	9,500,000	9,483,000
Platelets	907,100	904,500

The figures obtained in both experiments indicate that no significant changes in the platelet count took place as a result of the vitamin deficiency. In the first experiment the limits in the platelet count were 758,000 to 920,000 on the first day, and 742,000 to 940,000 on the 90th. In the second, the limits were 847,000 to 980,000 on the first day, and 841,000 to 976,000 on the 90th. The slight diminution observed falls well within the limits of normality or of experimental error, and it would seem that these results lend some support to the opinion of Bedson and Zilva. There was, in each experiment, an undoubted vitamin deficiency, and it is probable that this was more severe than in the animals examined by Bedson and Zilva and by Cramer, Drew and Mottram in view of the length of time the animals were kept on the deficient diet. There was no possibility that platelets could be mistaken for any of the bodies mentioned by the latter authors, and their technique was followed with the greatest care, so that it seems justifiable to conclude that the relatively enormous diminution in platelets observed by these authors cannot be regarded as a specific lesion due to avitaminosis, thus confirming the view of Bedson and Zilva.

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DIASTATIC ACTIVITY IN BLOOD AND URINE.

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As views on the utility of the estimation of diastase in urine and blood are divergent, an investigation into the diastase activity of equal quantities of whole blood and urine was undertaken. The diastatic activity of a specimen of urine is estimated in terms of the amount of starch which, incubated at 37° C. with a definite volume of the urine, will be changed in 30 minutes, the disappearance of the starch being indicated by the failure of the mixture of starch and urine to give a blue colour or a violet tint with iodine. For the estimation of the diastatic activity of blood, it is necessary to estimate the amount of sugar present in a given amount of blood, to incubate a given amount of blood with a given amount of starch at 37° C. for 30 minutes, and to estimate the amount of reducing sugar which has been formed by the diastase in the blood. The diastatic activity of the blood is given in terms of reducing sugar, and in this way a comparison can be made between the diastatic activities of equal volumes of blood and urine, although the actual concentration of the diastase by the kidneys would not be available.

Technique.

Urine.—In order to obtain the diastatic activity of equal volumes of blood and urine, a slight modification of the method described by Dodds (1922) was used.

A series of ten small test-tubes was employed usually, the length of the tube being about 3 inches, and capacity 4 c.c. A quantity of buffered urine was prepared by mixing 5 c.c. of the urine with 20 c.c. of a mixture of Sörensen's solutions. The buffer solution was obtained by mixing 15 c.c. of a solution containing 11·876 grm. $Na_2HPO_42H_20$ in 1000 c.c. distilled water, and 85 c.c. of a solution containing 9·078 grm. of KH_2PO_4 in 1000 c.c. distilled water. These solutions were kept in paraffin-coated glass-stoppered bottles. To each of the small ten test-tubes was added 1 c.c. of the buffered urine, and to the series of ten tubes (1–10) were added respectively 2 c.c., 1·8 c.c., 1·6 c.c., 1·4 c.c., 1·2 c.c., 1·0 c.c., 0·8 c.c., 0·6 c.c., 0·4 c.c., 0·2 c.c. of a 0·2 per cent. solution of Lintner's soluble starch made up in 0·9 per cent. NaCl solution. The total volume in each tube was made up to 3 c.c. by the addition of distilled water. The tubes were shaken immediately, and placed in a water-bath in an incubator for 30 minutes at 37°C. The tubes were then removed

from the incubator, and their contents poured into a series of larger test-tubes about three parts full of cold tap-water. One or two drops of a N/10 solution of iodine were added to the series of tubes (1–10) until a tube was obtained where the blue or violet tint was not perceptible. The amount of diastase in this tube was sufficient to digest all the starch present. The diastatic activity of the urine was found empirically by dividing the number of c.c. of the starch solution digested by the amount of urine in c.c., the number obtained often being called Wohlgemuth units. With the above technique the range of diastatic activities would be 20, 18, 16, 14, 12, 10, 8, 6, 4, 2—an even series of numbers not given by simple pipettings in previous methods. The range of diastatic activities could be easily increased by the use of stronger solutions of starch. For example, the use of a 0·4 per cent. solution of starch would give a range of diastatic activities from 40, 38, 36, . . . 24, 22, but the necessity for this was not common when the specimen of urine for examination was passed in the second two-hourly interval after the first meal of the day. This period was chosen because it appeared to be a convenient time to examine both the blood and urine, and because most of the diuresis due to the intake of fluid with the meal had ceased.

It was shown by Michaelis and Peckstein (1914) that the pH at which diastase was most active varied with the salt content of the medium. Stafford and Addis (1924) pointed out that in the technique of Dodds (*loc. cit.*) and Sladden (1922) there seemed to be a danger of a disturbing variation in the chloride and phosphate concentration respectively. This difficulty has been got over by the above modification, in which there was always in each tube a sufficiency of chloride and phosphate, and in which the urine with the enzyme was diluted equally in all the tubes, although the concentration of the substrate varied. This, however, would not prevent a reasonably accurate study of the diastatic activity of different urines.

In the present investigation the above modification was adopted, and was used before the publication of the second and third papers referred to in the preceding paragraph. Sladden (*loc. cit.*) considered that the addition of phosphate buffer solutions tended to obscure the final readings. But in the present inquiry no difficulty in reading the end-point was observed if small tubes (4 c.c.) were used for incubation, and if these were emptied into larger tubes (20–30 c.c.) containing cold water before the addition of iodine.

Blood.—For the examination of the diastase in the blood, the method described by G. Matthew Fyfe (1923) was employed with one or two small modifications.

Into one of two 100 c.c. Erlenmeyer flasks, 1·8 c.c. of Sörensen's buffer solutions and 1 c.c. of 0·1 per cent. solution of Lintner's soluble starch in 0·9 per cent. NaCl are pipetted, and into the other 23·8 c.c. of a 15 per cent. solution of sodium sulphate acidified by the addition of glacial acetic acid to the extent of 0·1 per cent. Into each flask is introduced 0·2 c.c. blood by means of two special pipettes which are thoroughly rinsed out in the clear fluid. The flask containing starch is placed in a water-bath in an incubator for 30 minutes at 37°C. At the end of this period 21 c.c. of the acid sodium sulphate solution are added immediately. The amount of sugar is estimated in both flasks by MacLean's method (1919).

The amount of sugar formed by 0·2 c.c. blood from 1 c.c. of a 0·1 per cent. solution of starch expressed in milligrammes is taken as an index of the diastatic activity of the blood. The number obtained multiplied by 100 to displace the decimal point is used as the number indicating the diastatic activity of the blood.

VARIATIONS IN THE TWENTY-FOUR-HOURLY SPECIMENS OF URINE.

With regard to 24-hourly specimens of urine, considerable variations were found from day to day under apparently constant conditions, the variations tending to be greater in the case of those individuals whose urine had a high diastatic activity.

Table I gives the variations (in Wohlgemuth units) obtained in a number of healthy individuals, specimens of the total urine passed in the 24 hours being examined.

TABLE I.

J. R—	5·4, 4·5, 6, 6, 3, 3·5, 3·8.
D. M—	9·1, 6, 10, 10, 10, 9.
C. G—	7·5, 9·7, 11, 8.
G. M—	20·8, 16·3, 16, 12·5, 15·4, 11·8, 13·2, 13·6, 10.
F. M—	7·2, 8·8, 6·5, 5·2, 5·7, 7·2, 5·8, 7·0, 5·5.
R—	19, 19, 20, 18·2, 15·4, 22·2, 22, 12·5, 22, 21, 16·7, 18, 12·5, 19, 20, 12·8.

The diastatic activity was not always directly related to the specific gravity or inversely to the average hourly rate of the secretion of urine, although it was found that the 24-hourly specimens with a higher specific gravity tended to have a higher diastatic value, while specimens with a lower rate of secretion tended to have a higher diastatic activity.

The total diastatic activity for the 24 hours was taken as the amount of urine in c.c. multiplied by the diastatic index stated in Wohlgemuth's units. This was found to give widely different numbers in different individuals. But by taking a large number of observations on the same individual over a considerable period, it was found that the total diastatic activity gave, in the majority of cases, a number which was fairly constant. For example:

In 9 readings on one individual, 6 gave a total diastatic content between 26,000–30,000.

In 9 readings on a second individual, 6 gave a total diastatic content between 12,000–16,000.

In 10 readings on a third individual, 8 gave a total diastatic content between 17,000–19,500.

In 15 readings on a fourth individual, 12 gave a total diastatic content between 13,000–16,000.

The remaining results of the individual cases were either above or below the respective numbers, the highest number obtained being as much as 50 per cent. above the lowest in the same case.

With regard to the individual specimens passed throughout the 24 hours,

considerable variations in the diastatic activity stated in Wohlgemuth units were met with. For example, variations 5–18, 9–22, 2–10, 2–6, 4–8, 12–24, etc., were obtained in different individuals.

The fewer the specimens, the less were the variations of the diastatic activity of the different specimens.

Conditions of polyuria induced by exitement, cold, drinking large quantities of fluids gave more striking variations. In one case it was noted that during excitement the diastatic activity of the urine fell from 20 to 1 in the course of two or three hours.

Stocks (1915–16) stated that it appeared that the concentration of diastase was at a maximum just after breakfast (8 a.m.), and then decreased gradually with a secondary rise after dinner.

Table II gives some observations made by the present writer on hospital patients who had breakfast at 7 a.m., dinner at 12 noon, tea at 4 p.m., supper at 7 p.m.

TABLE II.

	8.30 a.m.	11 a.m.	1.30 p.m.	5 p.m.	8 p.m.	5 a.m.
F. M—	8	5	5	14	5	12·5
G. M—	16·6	25	10	25	20	16·6
C. G—(1)	8	5	8	12·5	12·5	12·5
,, (2)	12·5	8	10	10	12·5	14·3
D. M—	8	6	—	10	10	14·3

The highest concentration of diastase in hospital patients who were not suffering from renal disease, and who were confined to bed, was obtained in the specimen of urine passed at 5 p.m. or in the overnight specimen passed at 5 a.m.

The observations in Table III were made on healthy young adults whose ordinary daily routine was not altered in any way.

TABLE III.—*Breakfast 8 a.m.*

Subject A.		Subject B.	
Diastatic activity of urine. (Wohlgemuth units.)	Time.	Diastatic activity of urine. (Wohlgemuth units.)	Time.
16·7	8.45 a.m.	14·3	8.45 a.m.
22·2	10.5 ,,	22·2	9.45 ,,
20	11.40 ,,	20	1.25 p.m.
18·2	1.10 p.m.	Meal.	
Meal.		15·4	3.45 ,,
16·7	4.40 ,,	12·5	4.30 ,,
20	6.15 ,,	20	5.40 ,,
Meal.		Meal.	
14·2	8.0 ,,	18·2	10.30 ,,
15·4	9.5 ,,	25	8.0 a.m.
18·2	12 midnight		
22·2	8.20 a.m.		

In neither of the above observations did the urine which was passed immediately after breakfast have the highest concentration of diastase. It would appear that the urine, which is secreted overnight, and which in healthy individuals is secreted at the slowest rate, has the highest diastatic activity, provided that factors producing polyuria were excluded. Specimens of urine were obtained before and immediately after breakfast, the first specimen being passed about 8 a.m. and the second about 8.45 a.m. to 9 a.m. The first specimen included the urine secreted overnight (Table IV).

TABLE IV.

Urine passed at 8 a.m. before breakfast.		Urine passed at 8.45 a.m. after breakfast.	
Diastatic activity.	Urine per hour in c.c.	Diastatic activity.	Urine per hour in c.c.
18·2	22	16·7	25
22·2	17	16·7	40
25	17	14·3	27
25	18	16·7	27
22·2	24	16·7	35
25	23	20	27
22·2	21	18·2	30

The lower diastatic activity of the second specimen evidently depends largely on the increased rate of secretion as compared with the relatively slow rate of secretion of night urine. On the other hand, it was found occasionally that the overnight urine had not the highest diastatic activity of specimens passed during the 24 hours' period. This can be seen on reference to Tables II and III.

The diastatic activity of the individual specimens passed during the 24 hours varied inversely as a rule with the average hourly rate of secretion. Exceptions, however, were rather frequent.

Table V gives the type of results obtained on a day during which no food or fluid was taken from 8 a.m. until evening.

TABLE V.

Time.	Amount in c.c.	Time after food in hours.	Diastatic activity. (Wohlgemuth units.)	Rate of secretion per hour in c.c.	Average hourly total diastatic activity.
7.30 a.m.	330	—	16	33	528
7.45 ,,	18	—	20	36	720
8.0 ,,	(breakfast)				
10.30 ,,	160	2½	16	58	932
11.35 ,,	102	3½	12	94	1132
1.5 p.m.	131	5	10	87	883
2.10 ,,	73	6¼	10	67	674
4.40 ,,	83	8¾	16	33	532
5.20 ,,	18	9¼	18	27	486

VARIATIONS IN DIASTATIC ACTIVITY OF BLOOD.

Wohlgemuth's method for diastase in the urine would appear not to be sufficiently delicate for serum, owing possibly to the concentration of diastase in the urine being higher than in the serum. The tint of the sera masks the delicacy of the colour reaction which occurs on the addition of iodine. Variations were found in the diastatic activity of whole blood when examined by Fyfe's method, and they would appear to be related to the ingestion of food.

Table VI shows the the variations obtained in two normal cases selected at random from a number of estimations made on several normal cases.

TABLE VI.

Case A.		Case B.	
Time.	Blood diastase.	Time.	Blood diastase.
7.30 a.m.	10·2	8 a.m.	6
Meal at 8 a.m.		Meal at 8.15 a.m.	
10.15 a.m.	11·9	9.15 a.m.	5
11.30 „	6·8	10.45 „	2·9
1.30 p.m.	6·3	1.0 p.m.	6·5
4.30 „	9·9	3.0 „	6·4
		5.0 „	5

Stafford and Addis (*loc. cit.*) foresaw the possibilities of variations in the diastatic activity of plasma, but they gave no details. Cammidge and Howard (1923) noted variations in the diastase content of the blood of a rabbit.

DIASTATIC ACTIVITIES OF URINE AND BLOOD, AND THE DIASTATIC CONCENTRATION FACTOR.

The diastatic activity of a specimen of urine has been shown to be—

$$\frac{\text{Number of c.c. of 0·2 per cent. solution of starch converted by 0·2 c.c. urine}}{\text{0·2 c.c. urine}}$$

$$= \frac{\text{number of milligrammes of starch converted}}{\text{0·2 c.c. urine}}$$

$$i.\,e.\ \text{U.D.} = \frac{\text{number of milligrammes of starch}}{\text{0·2.}}$$

The diastatic activity of the blood (B.D.) has been taken as the number of milligrammes of sugar × 100 formed by the diastase in 0·2 c.c. blood from the substrate, viz. starch.

The diastatic concentration factor (D.C.F.) for the kidneys which are being examined may be taken as the power of the kidneys to concentrate diastase from the blood. If, for example, a specimen of urine is obtained for the second two-hourly period after the first meal of the day, in order to get the mean value of the blood diastase during this period, it would be necessary to carry out blood-diastase estimations at the beginning and end of this period or

alternatively at the middle of this period. As it is impossible to estimate actually the amount of diastase in the urine and blood, the ratio of the amount of starch converted by 0·2 c.c. urine to the amount of sugar formed by 0·2 c.c. blood has been taken as the diastatic concentration factor.

$$\text{D.C.F.} = \frac{\text{number of milligrammes of starch converted by 0·2 c.c. urine}}{\text{number of milligrammes of sugar formed by 0·2 c.c. blood}}$$

$$= \frac{\text{U.D.} \times 0{\cdot}2}{\frac{\text{B.D.}}{100}}$$

$$= \frac{\text{U.D.} \times 0{\cdot}2}{\text{B.D.}} \times 100.$$

The diastatic concentration factor was found to vary considerably throughout the 24 hours. The results in Table VII from two subjects—typical of a series of at least half-a-dozen—show the sort of variations obtained in a number of individuals.

TABLE VII.—*Breakfast* 8 *a.m.*

	Time.	B.D.	U.D.	D.C.F.
Case A:	7.30 a.m.	10·2	16	30 +
	10.15 „	11·9	16	29
	11.30 „	6·8	12	25
	1.30 p.m.	6·3	10	30
	4.30 „	9·9	16	40
Case B:	8.0 a.m.	6	6	20 approximately.
	9.15 „	5	—	—
	10.45 „	2·9	8	36
	1.0 p.m.	6·5	6	25
	3.0 „	6·4	8	25
	5.0 „	5	8	28

From the point of view of convenience it was decided to carry out a number of investigations on healthy adults, and to examine the diastatic activity of the blood at two hours and four hours or alternatively at three hours after the first meal of the day, and to examine the diastatic activity of the urine secreted during the second two-hourly period after the same meal.

Table VIII gives the diastatic concentration figures obtained in young adults and in children of fifteen and under by the above method.

A factor which must be considered in cases giving low urinary diastatic figures, apart from those due to polyuria, is that of the blood-diastase figure.

Case 6 has a low U.D. and moderately low B.D., but the lowness of the D.C.F. is due to the presence of a certain amount of diuresis due to excitement. Case 14, on the other hand, shows low figures for the U.D. and B.D., but the D.C.F. in the absence of polyuria is within normal limits.

It will be noted that if due allowance is made for variations due to polyuria, the figure obtained from these normal individuals examined for the diastatic concentration factor lies between 15 and 40.

TABLE VIII.

	Sex.	Age.	U.D.	B.D.	D.C.F.	Amount of urine during two hours.
1.	M.	31	16	9·4	29	110 c.c.
2.	M.	25	12	8·1	29	65 „
3.	M.	25	6	4·7	25	175 „ (usually passes large quantity).
4.	M.	29	12	6·4	37	115 „
5.	M.	9	6	8	15	80 „ sp. gr. 1020.
6.	M.	5	2	5	8	155 „ „ 1010; polyuria.
7.	M.	9	6	8	15	125 „ „ 1014.
8.	M.	11	8	7	23	90 „ „ 1021.
9.	M.	10	2	8	5	275 „ „ 1010; polyuria.
10.	M.	10	2	5	8	240 „ „ 1010; „
11.	F.	11	8	8	20	55 „
12.	F.	7½	8	8	20	35 „
13.	F.	6½	6	5	24	130 „ „ 1018.
14.	M.	5¾	4	3	27	75 „ „ 1022.
15.	M.	13	6	10	12	110 „
16.	M.	13	4	10	8	160 „ — polyuria.
17.	M.	14	8	11	15	70 „
18.	M.	13	6	9	14	70 „

SUMMARY OF RESULTS.

Healthy Subjects.

I. *Diurnal variations in the urine.*—The urine secreted during the night has generally a higher diastatic activity than that secreted during the day. The diastatic activity of the urine secreted during the day varies inversely, as a rule, with the rate of excretion, and directly to a certain extent with the specific gravity. The total diastatic activity of the urine (amount in c.c. × diastatic index) for the 24 hours gives numbers only approximately constant in the same individual. The total diastatic activity of specimens of urine examined hourly after a meal is highest in the period of the second to the fifth hour. Thereafter the value tends to fall.

II. *Variations in the blood.*—The blood diastase when examined by Fyfe's method exhibits well-marked variations apparently related to the ingestion of food.

The level is lower in the period from the third or fourth hour to the seventh or eighth hour after a meal than it is after fasting or shortly after a meal.

III. *Variations in the diastatic concentration factor.*—In the same individual this varies throughout the day, being highest, as a rule, in the urine secreted overnight or in urine secreted during the fasting condition.

IV. *The figures obtained for the period 2–4 hours after a meal in healthy individuals:* (1) *Blood diastase.*—In the majority the figure obtained lies between 6 and 10, with outside limits of from 3 (exceptionally) to 11 or 12.

TABLE IX.

Case.	Breakfast. a.m.	Time. a.m.	B.D.	Time. a.m.	B.D.	Urine amount in c.c. 6.0–8.0 a.m.	U.D.	Urine amount in c.c. 8.0–10.0 a.m.	U.D.	D.C.F.	Remarks.
1 M. 26:											
(1) 8 . 8 . 23	6.30	8.30	12	10.35	5·5	—	—	—	—	—	Albumen 12 %. Œdema.
(2) 10 . 8 . 23	6.30	8.30	5	10.50	7·6	35	18	110	18	57	Albumen 12 %. Improved under urea and high protein diet.
(3) 4 . 9 . 23	6.0	8.15	8·4	10.30	10	120	12	150	10	26	Albumen 7 %.
(4) 17 . 9 . 23	7.40	10.40	15	—	—	—	—	—	—	—	Albumen 6 %. Recovery good—one year later.
2 M. 55 . .	7.0	9.5	7	11.5	3·5	—	—	100	8	30	Albumen, trace; deposit phosphates.
3 M. 51 . .	6.0	8.0	10·2	10.20	9·1	—	—	—	14	29	No albumen; previous history of albuminuria.
4 F. 22 . .	6.0	8.10	13·2	10.15	16·1	100	18	150	12	16·5	Eclampsia. Albumen + granular casts. Recovery.
5 F. 34 . .	6.0	8.15	11·9	10.40	7·9	300	2	100	12	24	Albumen, small quantity; occasional cast. Health good.
6 F. 55 . .	6.0	8.15	6·2	10.30	6	45	8	90	12	38	Albumen, small amount; granular casts; few blood-cells.
7 F. 30 . .	6.0	8.15	7·8	10.30	9·2	105	4	90	4	9·4	Puerperal septicæmia. Albumen + blood-cells; occasional cast. Recovery.
8 M. 50 . .	6.0	8.20	10·5	10.10	7·3	45	10	165	12	27	Albumen, trace; occasional granular and cellular cast.
9 F. 30 . .	6.0	8.0	4·5	10.5	8·2	—	—	75	8	25	Eclampsia. Albumen, small amount; few blood-cells; hyaline and granular casts.
10 F. 68 . .	6.0	8.0	12·8	10.15	11·8	180	—	40	18	29	Occasional casts; numerous blood-cells. (Auricular fibrillation.)
11 M. 60 . .	6.0	8.10	10	10.10	9	240	8	150	8	17	Albumen + + numerous casts and R.B.Cs.; arterio-venous aneurysm forearm. Wassermann R. + +.
12 F. 60 . .	6.0	8.15	7	10.30	9·4	—	—	105	16	39	Albumen, small amount; occasional R.B.C.; bronchitis.
13 M. 63 . .	6.0	8.15	17·3	10.0	12·9	120	14	240	10	13·3	Aortic case. Albumen, trace. Died two days later, suddenly.
14 F. 73 . .	6.0	8.10	10	10.25	14·3	180	10	165	4	5	Albumen, trace. Auricular fibrillation and C.S. breathing. Died shortly after.
15 M. 57 . .	6.0	8.0	17·8	10.15	10	18	16	—	16	23	Albumen 2–4 %; R.B.Cs. and casts. Œdema present.
16 M. 73:											
(1) 8 . 8 . 23	7.0	8.55	4	11.0	3	—	—	240	10	67	Albumen, small amount. Malignant

16 M. 73:											
(1) 11 . 9 . 23	7.0	8.55	4	11.0	2	—	—	240	10	67	Albumen, small amount. Malignant disease of liver and lungs. Died 14 . 9 . 23.
(2) 13 . 9 . 23	7.0	8.55	18	—	—	—	—	—	—	—	—
17 F. 40 . .	7.30 (fluids)	8.40	11·5	11.0	11·8	—	—	270	2	3·5	Hæmaturia of several weeks. Large amount of blood. Albumen; cells.
18 M. 52 . .	6.30 12.30 p.m. (50 gm. lævulose)	12.20 p.m.	4	1.10 p.m. 2.5 p.m. 3.50 p.m.	11·5 11·8 14·5	—	—	—	—	—	Sugar percentage: ·109, ·104, ·109, ·102.
19 M. 45 . .	8.30 (50 gm. lævulose)	9.30 a.m.	6·5	10.40 a.m. 11.40 a.m. 12.40 p.m.	5 3·6 3·6	—	—	—	—	—	Sugar percentage: ·128, ·162, ·137, ·131.
20 F. 55 . .	6.0 (Quaker oats)	8.15	7	10.20 a.m.	7·6	60	6	180	4	11	Albumen, trace; occasional cast. Percentage sugar in blood: ·312 and ·236.
21 F. 56 . .	7.30 (ord. diet)	9.10	8·8	11.40	4·4	—	—	—	6	18	Urine 12 % sugar; percentage in blood: ·38, ·46, ·468.
22 M. 38 . .	—	—	—	—	22	—	—	—	12	11	Blood-sugar 0·2 %; urine sugar ½ %.
23 M. 30 . .	—	—	—	—	15	—	—	—	6·6	9	Blood-sugar 0·12 %; urine sugar ½ %.
24 M. 29 . .	—	—	—	—	4	—	—	—	10	50	Blood-sugar 0·11 %; urine sugar ½ %.
26 F. 11 . .	—	—	—	10.0	7	—	—	9–11 a.m. —	<2	3	Sp. gr. 1011; 135 c.c. Very few blood-cells; occasional hyaline and granular casts.
27 F. 13 . .	—	—	—	10.0	9·2	—	—	—	4·5	10	Sp. gr. 1028; 65 c.c. Numerous R.B.Cs.; occasional granular casts.
28 M. 10 . .	—	—	—	10.0	14	—	—	—	2	3	Sp. gr. 1015; 175 c.c. Numerous R.B.Cs; occasional granular casts.
29 M. 18 . .	—	—	—	10.0	12	—	—	—	12	20	Sp. gr. 1030; 80 c.c. Some R.B.Cs.; very numerous casts. Recovering from uræmia.
30 M. 15:											
(1) 8 . 12 . 23	—	—	—	10.0	6	—	—	—	20	66	Very scanty urine with a large quantity of blood. Œdema present.
(2) 20 . 12 . 23	—	—	—	„	15	—	—	—	12	16	Sp. gr. 1022; 35 c.c. R.B.Cs. Numerous casts of all kinds. Small quantity of blood.
31 M. 46 . .	—	—	—	„	8	—	—	—	<2	<5	Arterio-sclerosis; retinal hæmorrhages.
32 F. 42 . .	—	—	—	„	4	—	—	—	3	15	High blood-pressure; small amount of sugar in urine.
33 M. 68 . .	—	—	—	„	5·5	—	—	—	6	22	Auricular fibrillation and arterio-sclerosis.
34 F. 50 . .	—	—	—	„	20	—	—	—	3	3	Arterio-sclerosis.
35 M. 50 . .	—	—	—	„	8	—	—	—	7	18	Chronic interstitial nephritis.
36 F. 47 . .	—	—	—	„	10	—	—	—	8	16	Hyperpiesis.
37 F. 40 . .	—	—	—	„	9	—	—	—	20	44	Acute nephritis with œdema.

(2) *Urine diastase.*—In the absence of polyuria the diastatic index in the majority of healthy individuals lies between 6 and 14.

(3) *Diastatic concentration factor.*—The normal figure obtained lies in the majority of cases between 20 and 30 with outside limits of about 15 to 40.

Nephritic Subjects, etc.

Numerous cases of nephritis, mostly, of diabetes, arteriosclerosis, prostatic enlargement, etc., were examined in the same way as the preceding normal cases with a view to seeing whether there was any variation from the normal diastatic concentration factor in renal disease.

The results are shown in Table IX.

Blood diastase.—High levels were found in a number of kidney conditions such as acute nephritis, uræmia, cardio-renal cases with failing heart. In addition, high levels were also found in 2 out of 5 diabetic cases and in 2 out of 4 cases of prostatic enlargement with retention of urine.

Urine diastase.—High levels were found in acute nephritis and in cases of cardio-renal disease showing failure of urinary secretion. Low levels were found in many cases of chronic kidney disease and of prostatic enlargement with retention.

Diastatic concentration factor.—While the figures obtained in acute nephritis were high, low figures were obtained in cases of chronic renal disease, enlarged prostate and diabetes.

Prostatic Cases.

Table X shows the results obtained in four cases of prostatic enlargement with retention of urine.

TABLE X.

Case.	Age.	Blood urea.	Urine urea.	B.D.	U.D.	D.C.F.	Remarks.
38	65	90	—	11·4	2	3·5	General condition fair.
39	85	150	1·35%	3	<2	Low	General condition poor; chronic uræmic state.
40	79	60	1·2%	7	<2	„	Pus in urine; clearing; general condition improving.
41	63	90	1·6%	16	6	7	Stricture and enlarged prostate; blood and pus in urine; tongue dry; general condition poor.

Wide variations were observed in the blood-diastase figure. The urine diastase figures were low with one exception. All the cases, however, gave a low diastatic concentration factor, as the above-mentioned exception (Case 41) had a high blood-diastase figure.

Urea Concentration and Diastatic Concentration Factor.

In the cases shown in Table XI the urea concentration test was performed and a comparison was made with the diastatic concentration factor.

TABLE XI.

Age.	Sex.	Urea concentration.	D.C.F.	Remarks.
30	M.	2·4%	24	Healthy.
25	M.	2·5%	22	,,
46	M.	1·25%	5	Arterio-sclerosis with retinal hæmorrhages.
42	F.	2·4%	15	Hyperpiesis.
63	M.	3·0%	22	Auricular fibrillation.
47	F.	0·75%	3	Chronic interstitial nephritis.
50	M.	2·0%	18	,, ,,
47	F.	3%–3·5%	16	Hyperpiesis.
40	F.	4·3%	44	Acute nephritis.
85	M.	1·3%	5	Enlargement of prostate.
79	M.	1·2%	5	,, ,,
63	M.	1·6%	7	,, ,,
81	M.	3·05%	25	,, ,,

The figure obtained in healthy individuals for the diastatic concentration factor lies between 15 and 40. When the figures in the two columns for the urea concentration and the diastatic concentration factor are compared they are found to be in general agreement, normal values of one corresponding to normal percentages of the other, and low values of one corresponding to low percentages of the other.

Stafford and Addis (*loc. cit.*) compare the rate of excretion of diastase with the power of the kidney to concentrate urea, and state as a remarkable fact that emphasis has usually been laid on the concentration of diastase, and not on the rate of excretion of diastase. They obtain the rate of excretion or the total hourly diastatic activity by multiplying the amount of urine in c.c. by the urinary diastatic activity (Wohlgemuth units), and dividing by the time in hours which the kidney took to secrete the specimen considered. In MacLean's urea concentration test stress is laid on the power of the kidney to concentrate urea. In the present investigation stress is laid on the power of the kidney to concentrate diastase from the blood, and not on the rate of excretion as shown by the total hourly diastatic activity.

It is possible to have (*cf.* Case 13, Table IX) a normal urinary diastatic activity and total hourly diastatic activity along with a high blood diastatic activity, thus producing a diastatic concentration factor lower than normal. Further, the hourly rate of excretion is found to vary so widely in different normal individuals that the application of the hourly rate of excretion of diastase to abnormal cases would not appear to be justifiable.

In addition, in the present investigation the diastatic concentration factor is compared in many cases with the urea concentration as obtained by MacLean's test, and in this way conclusions are avoided that depend on a comparison between the urea concentration and the hourly rate of excretion of diastase, which is very variable even in normal subjects.

CONCLUSIONS.

It is advisable to estimate the blood diastatic activity in all cases where the urine diastatic activity is being examined, and especially in those cases which give a urinary diastase figure towards the lower limits of normality.

It would appear that the diastatic concentration factor would serve as an additional confirmatory test to MacLean's urea concentration test, as the figures obtained for both tests in healthy and pathological cases were in general agreement.

I am indebted to Prof. J. A. MacWilliam and to Prof. H. MacLean for much kindly help and suggestion, and to Dr. W. Brander, Medical Superintendent, Hackney Infirmary, for permission to examine numerous cases.

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